

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

IRVINE, Jonquil, Claire
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5LX
ROYAUME-UNI

Date of mailing (day/month/year)

20 February 2002 (20.02.02)

Applicant's or agent's file reference

N.82592 JCI

IMPORTANT NOTIFICATION

International application No.

PCT/GB00/03800

International filing date (day/month/year)

04 October 2000 (04.10.00)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address

WALTON, Sean, M.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
United Kingdom

State of Nationality

State of Residence

Telephone No.

020 7240 4405

Facsimile No.

+44 20 7240 9339

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

IRVINE, Jonquil, Claire
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5LX
United Kingdom

State of Nationality

State of Residence

Telephone No.

+44 207 205 3292

Facsimile No.

+44 207 212 8932

Teleprinter No.

3. Further observations, if necessary:

A sub-agent has been appointed and the file reference changed to the one indicated in the box above.

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Antonia MULLER

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

CWK N:82592

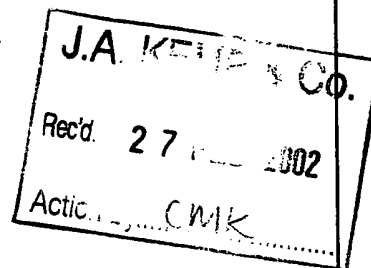
From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

IRVINE, Jonquil, Claire
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5LX
ROYAUME-UNI



Date of mailing (day/month/year) 20 February 2002 (20.02.02)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference SMW/BP5884200	
International application No. PCT/GB00/03800	International filing date (day/month/year) 04 October 2000 (04.10.00)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address

ISIS INNOVATION LIMITED
Ewert House
Ewert Place
Summertown
Oxford OX2 7DD
United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

ISIS INNOVATION LIMITED
Ewert House
Ewert Place
Summertown
Oxford OX2 7SG
United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Genève 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Antonia MULLER

Telephone No.: (41-22) 338.83.38

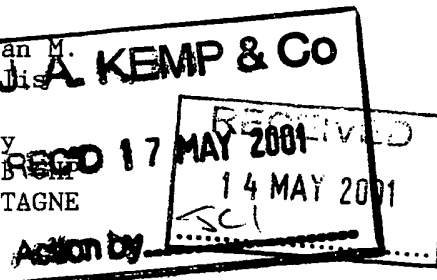
TENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

WALTON, Sean M.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6SM
GRANDE BRETAGNE



NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing
(day/month/year)

10-05-2001

Applicant's or agent's file reference

SMW/CP5884200

IMPORTANT NOTIFICATION

International application No.

PCT/GB 00/ 03800

International filing date (day/month/year)

04/10/2000

Priority date (day/month/year)

04/10/1999

Applicant

ISIS INNOVATION LIMITED et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

24/04/2001

2. This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- ☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- ☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

- ☐ (If applicable) This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

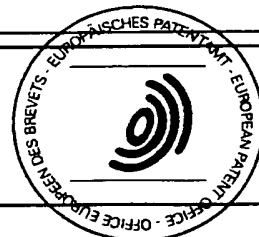


European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Netherlands
Tel.: (+ 31-70) 340-2040
Fax: (+ 31-70) 340-3016

Authorized officer

CARDENAS C E

Tel. (+ 31-70) 340-3370



PCT

**INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION**

(PCT Rule 61.3)

From the INTERNATIONAL BUREAU

To:

IRVINE, Jonquil, Claire
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5LX
ROYAUME-UNI

J.A. KEMP & Co.
Rec'd. **27 FEB 2002**
Action by... CMK.....

Date of mailing (day/month/year) 20 February 2002 (20.02.02)		
Applicant's or agent's file reference SMW/BP5884200		IMPORTANT INFORMATION
International application No. PCT/GB00/03800	International filing date (day/month/year) 04 October 2000 (04.10.00)	
		Priority date (day/month/year) 04 October 1999 (04.10.99)
Applicant ISIS INNOVATION LIMITED et al		

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP : AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
National : AU, BG, CA, CN, DE, GB, IL, JP, KP, KR, MN, NO, NZ, PL, RO, RU, SE, SK, US


2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

AP : GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW
EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
OA : BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
National : AE, AG, AL, AM, AT, AZ, BA, BB, BR, BY, BZ, CH, CR, CU, CZ, DK, DM, DZ, EE, ES, FI,
GD, GE, GH, GM, HR, HU, ID, IN, IS, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MW,
MX, MZ, PT, SD, SG, SI, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

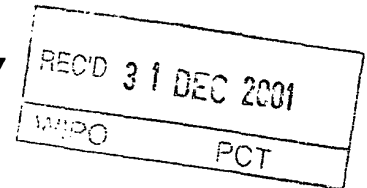
Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

<p>The International Bureau of WIPO 34, chemin des C lombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No. (41-22) 740.14.35</p>	<p>Authorized officer:</p> <p>Antonia MULLER </p> <p>Telephone No. (41-22) 338.83.38</p>
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PATENT COOPERATION TREATY

PCT



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference N.82592 JCI		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB00/03800	International filing date (day/month/year) 04/10/2000	Priority date (day/month/year) 04/10/1999	
International Patent Classification (IPC) or national classification and IPC C12N15/85			
Applicant ISIS INNOVATION LIMITED et al.			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 6 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>			
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none">I <input checked="" type="checkbox"/> Basis of the reportII <input type="checkbox"/> PriorityIII <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicabilityIV <input type="checkbox"/> Lack of unity of inventionV <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statementVI <input checked="" type="checkbox"/> Certain documents citedVII <input type="checkbox"/> Certain defects in the international applicationVIII <input type="checkbox"/> Certain observations on the international application			
Date of submission of the demand 24/04/2001		Date of completion of this report 27.12.2001	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized officer Cupido, M Telephone No. +31 70 340 3374	



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/03800

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):
Description, pages:

1-49 as originally filed

Claims, No.:

1-52 as originally filed

Drawings, sheets:

1/15-15/15 as originally filed

Sequence listing part of the description, pages:

1-36, filed with the letter of 06.12.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
☒ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/03800

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:
5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:
- ☐ the entire international application.
- ☒ claims Nos. 35 and 39.

because:

- ☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the said claims Nos. .
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/03800

1. Statement

Novelty (N)	Yes:	Claims	1,10-22,28-35,37,38,40-52
	No:	Claims	2-9,23-27
Inventive step (IS)	Yes:	Claims	1,10-22,28-35,37,38,40-52
	No:	Claims	2-9,23-27
Industrial applicability (IA)	Yes:	Claims	1-35,37,38,40-52
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 36 and 39 are directed to a method of treatment of the human or animal body, see Article 34(4)(a)(I) and Rule 67.1(iv) PCT.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

I Documents

The following documents have been taken into consideration:

D1: EMBL Database sequence HS91J24

D2: WO-A-97/22696 (Medical Research Council)

II Novelty

1. Nucleotides 87734-88930 from sequence HS91J24 are identical to the human promoter sequence shown in figure 1. This sequence also comprises a part of the utrophin coding sequence, and hence the subject-matter of claim 1 is regarded to be novel. However, the proviso that the claimed sequence should be free of utrophin coding sequences that makes claim 1 novel is not present in claims 2-22. Since claims 10-22 and 30-34 refer to constructs containing heterologous sequences, the subject-matter in these claims is also regarded to be novel. However, the subject-matter in claims 2-9 and also in claims 23-27 is regarded to lack novelty in view of Article 33(2) PCT.

2. The subject-matter of claims 28, 29, 35, 37, 38 and 40-52 was not disclosed in the prior art.

III Inventive step

1. D2 is regarded as the closest prior art with respect to the question whether the claimed subject-matter involves an inventive step. D2 discloses a promoter at the 5'

end of the utrophin locus and its application for expression of mini-genes and chimeric constructs, and their potential use in therapeutic methods to treat Duchenne muscular dystrophy (DMD). The problem underlying the present invention in view of D2 is the provision of further nucleic acid constructs that may be used to treat DMD.

2. The solution provided and claimed in the present application concerns another promoter located in the second intron of the utrophin gene. The presence and the possible use of this promoter could not be derived from the prior art and hence the subject-matter in claims 1, 10-22, 29-35, 37, 38 and 39-52 is regarded to involve an inventive step as required by Article 33(3) PCT.

The present opinion has been established with the assumption that all the claims enjoy the claimed priority date of 4 October 1999. In this respect, the documents EP-A-1033401 and Burton et al, PNAS 96 14025-14030, cited in the International Search Report, has not been considered to be part of the prior art as defined in Rule 64 (1)-(3) PCT.

Re Item VI

Certain documents cited

Although EP-A-1 033 401 does not constitute prior art within the meaning of Rule 64.1(b), it appears to disclose all the features of claims 23-28. No check has been made as to whether the priority of this prior application has been validly claimed.

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only	
Identification of IPEA	Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION	
Applicant's or agent's file reference SMW/CP5884200	
International application No. PCT/GB/00/03800	International filing date (day/month/year) 4 october 2000 (04.10.2000)
(Earliest) Priority date (day/month/year) 4 October 1999 (04.10.1999)	
Title of invention UTROPHIN GENE PROMOTER	
Box No. II APPLICANT(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
ISIS INNOVATION LIMITED Ewert House Ewert Place Summetown Oxford OX2 7SG UNITED KINGDOM	
Telephone No.:	
Facsimile No.:	
Teleprinter No.:	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
BURTON, Edward University of Pittsburgh School of Medicine Department of Molecular Genetics and Biochemistry E1215 Biomedical Science Tower Pittsburgh, Pennsylvania 15261 UNITED STATES OF AMERICA	
State (that is, country) of nationality: GB	State (that is, country) of residence: US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
TINSLEY, Jonathon 5 Spruce Gardens Oxford OX4 7GH UNITED KINGDOM	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
<input checked="" type="checkbox"/> Further applicants are indicated on a continuation sheet.	

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet is not to be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DAVIES, KAY
55 Five Mile Drive
Oxford OX2 8HR
UNITED KINGDOM

State (i.e. country) of nationality:
GB

State (i.e. country) of residence:
GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

☐ Further applicants are indicated on another continuation sheet

Box No. III AGE^{*} OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative
and ☐ has been appointed earlier and represents the applicant(s) also for international preliminary examination
☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Seán M Walton and Others
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
GB

Telephone No.:
020 7240 4405

Facsimile No.:
020 7240 9339

Teleprinter No.:

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

- 1 The applicant wishes the international preliminary examination to start on the basis of:
 - ☒ the international application as originally filed
 - the description ☒ as originally filed
☐ as amended under Article 34
 - the claims ☒ as originally filed
☐ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34
 - the drawings ☒ as originally filed
2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.
3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examination Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed, or where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

- ☒ which is the language in which the international application was filed
- ☐ which is the language of a translation furnished for the purposes of international search.
- ☐ which is the language of publication of the international application
- ☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination

- | | |
|---|--------|
| 1. translation of international application : | sheets |
| 2. amendments under Article 34 : | sheets |
| 3. copy (or, where required, translation) of
amendments under Article 19 : | sheets |
| 4. copy (or, when required, translation) of
statement under Article 19 : | sheets |
| 5. letter : | sheets |
| 6. other (<i>specify</i>) : | sheets |

For International Preliminary
Examining Authority use only
received not received

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

The demand is also accompanied by the item(s) marked below:

- | | |
|---|--|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 4. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> separate signed power of attorney | 5. <input type="checkbox"/> nucleotide and or amino acid sequence listing in
computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney;
reference number, if any: | 6. <input type="checkbox"/> other (<i>specify</i>): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

.....
Seán M Walton

APPOINTED AGENT

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due
to CORRECTIONS under Rule 60.1(b):

- | | |
|---|--|
| 3. <input type="checkbox"/> The date of receipt of the demand is AFTER the expiration of 19 months from
the priority date and item 4 or 5, below, does not apply. | <input type="checkbox"/> The applicant has been
informed accordingly. |
| 4. <input type="checkbox"/> The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5 | |
| 5. <input type="checkbox"/> Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is
EXCUSED pursuant to Rule 82. | |

For International Bureau use only

Demand received from IPEA on:



PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 20 February 2002 (20.02.02)	
International application No. PCT/GB00/03800	Applicant's or agent's file reference SMW/BP5884200
International filing date (day/month/year) 04 October 2000 (04.10.00)	Priority date (day/month/year) 04 October 1999 (04.10.99)
Applicant BURTON, Edward et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

24 April 2001 (24.04.01)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Antonia MULLER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (if desired) (12 characters maximum) SMW/BP5884200

Box No. I TITLE OF INVENTION Promoting Gene Expression

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

ISIS Innovation Limited
Ewert House
Ewert Place
Summertown
Oxford OX2 7DD
UNITED KINGDOM

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (that is, country) of nationality: GB

State (that is, country) of residence: GB

This person is applicant for the purposes of:

☐ all designated States

☒ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

BURTON Edward
University of Pittsburgh
School of Medicine
Department of Molecular Genetics and Biochemistry
E1215 Biomedical Science Tower
Pittsburgh
Pennsylvania 15261
UNITED STATES OF AMERICA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality: GB

State (that is, country) of residence: US

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent

☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

WALTON, SEÁN M. and others
MEWBURN ELLIS
YORK HOUSE
23 KINGSWAY
LONDON WC2B 6HP
GB

Telephone No. 020 7240 4405

Facsimile No. +44 20 7240 9339

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

TINSLEY Jonathon
5 Spruce Gardens
Oxford OX4 7GH
UNITED KINGDOM

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality: GB

State (that is, country) of residence: GB

This person is applicant for the purposes of:

☐ all designated states

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

DAVIES Kay
55 Five Mile Drive
Oxford OX2 8HR
UNITED KINGDOM

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality: GB

State (that is, country) of residence: GB

This person is applicant for the purposes of:

☐ all designated states

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

☐ all designated states

☐ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only

☐ applicant and inventor

☐ inventor only (if this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

☐ all designated states

☐ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

- ☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection desired, specify on dotted line):

- | | |
|---|---|
| <input checked="" type="checkbox"/> AE United Arab Emirates | <input checked="" type="checkbox"/> LC Saint Lucia |
| <input checked="" type="checkbox"/> AG Antigua and Barbuda | <input checked="" type="checkbox"/> LK Sri Lanka |
| <input checked="" type="checkbox"/> AL Albania..... | <input checked="" type="checkbox"/> LR Liberia..... |
| <input checked="" type="checkbox"/> AM Armenia..... | <input checked="" type="checkbox"/> LS Lesotho..... |
| <input checked="" type="checkbox"/> AT Austria..... | <input checked="" type="checkbox"/> LT Lithuania..... |
| <input checked="" type="checkbox"/> AU Australia..... | <input checked="" type="checkbox"/> LU Luxembourg..... |
| <input checked="" type="checkbox"/> AZ Azerbaijan..... | <input checked="" type="checkbox"/> LV Latvia..... |
| <input checked="" type="checkbox"/> BA Bosnia & Herzegovina..... | <input checked="" type="checkbox"/> MA Morocco..... |
| <input checked="" type="checkbox"/> BB Barbados..... | <input checked="" type="checkbox"/> MD Republic of Moldova |
| <input checked="" type="checkbox"/> BG Bulgaria..... | <input checked="" type="checkbox"/> MG Madagascar |
| <input checked="" type="checkbox"/> BR Brazil..... | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia..... |
| <input checked="" type="checkbox"/> BY Belarus..... | <input checked="" type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> BZ Belize..... | <input checked="" type="checkbox"/> MW Malawi..... |
| <input checked="" type="checkbox"/> CA Canada..... | <input checked="" type="checkbox"/> MX Mexico..... |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein..... | <input checked="" type="checkbox"/> MZ Mozambique..... |
| <input checked="" type="checkbox"/> CN China..... | <input checked="" type="checkbox"/> NO Norway |
| <input checked="" type="checkbox"/> CR Costa Rica..... | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> CU Cuba..... | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> CZ Czech Republic..... | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> DE Germany..... | <input checked="" type="checkbox"/> RO Romania..... |
| <input checked="" type="checkbox"/> DK Denmark..... | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> DM Dominica..... | <input checked="" type="checkbox"/> SD Sudan..... |
| <input checked="" type="checkbox"/> DZ Algeria..... | <input checked="" type="checkbox"/> SE Sweden..... |
| <input checked="" type="checkbox"/> EE Estonia..... | <input checked="" type="checkbox"/> SG Singapore..... |
| <input checked="" type="checkbox"/> ES Spain..... | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> FI Finland..... | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> GB United Kingdom..... | <input checked="" type="checkbox"/> SL Sierra Leone..... |
| <input checked="" type="checkbox"/> GD Grenada..... | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> GE Georgia..... | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> GH Ghana..... | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> GM Gambia..... | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> HR Croatia..... | <input checked="" type="checkbox"/> TZ United Republic of Tanzania..... |
| <input checked="" type="checkbox"/> HU Hungary..... | <input checked="" type="checkbox"/> UA Ukraine |
| <input checked="" type="checkbox"/> ID Indonesia..... | <input checked="" type="checkbox"/> UG Uganda |
| <input checked="" type="checkbox"/> IL Israel..... | <input checked="" type="checkbox"/> US United States of America..... |
| <input checked="" type="checkbox"/> IN India..... | <input checked="" type="checkbox"/> UZ Uzbekistan |
| <input checked="" type="checkbox"/> IS Iceland..... | <input checked="" type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> JP Japan..... | <input checked="" type="checkbox"/> YU Yugoslavia |
| <input checked="" type="checkbox"/> KE Kenya..... | <input checked="" type="checkbox"/> ZA South Africa..... |
| <input checked="" type="checkbox"/> KG Kyrgyzstan..... | <input checked="" type="checkbox"/> ZW Zimbabwe |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea..... | |
| <input checked="" type="checkbox"/> KR Republic of Korea..... | |
| <input checked="" type="checkbox"/> KZ Kazakhstan | |

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ Any other state which is party to the PCT

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

U is box in the following cases:

1. *If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient; in particular:*
 - (i) *if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*
 - (ii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*
 - (iii) *if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*
 - (iv) *if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
 - (v) *if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
 - (vi) *if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
 - (vii) *if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*
2. *If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each state so excluded.*
3. *If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty; in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.*

Continuation of Box IV

ARMITAGE, IAN M.	WALTON, SEÁN M.
BRASNETT, ADRIAN H.	WATSON, ROBERT J.
CALDERBANK, T. ROGER	
CARTER, STEPHEN	
COLEIRO, RAYMOND	
CRIPPS, JOANNA E	
FORD, MICHAEL F.	
HACKNEY, NIGEL J.	
HARRISON, DAVID C.	
KIDDLE, SIMON J.	
KREMER, SIMON M.	
LYONS, JUNE, M.	
NICHOLLS, KATHRYN M.	
PAGET, HUGH C.E.	
SANDERSON, MICHAEL J.	
STONER, G. PATRICK	
STUART, IAN	

Box No. VI		PRIORITY CLAIM		<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box	
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:			
		national application: country	regional application:* regional Office	international application: receiving Office	
item (1) 4 October 1999 04/10/99)	9923423.9	GB			
item (2)					
item (3)					

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): (1)

* Where the earlier application is an ARIPO application, it is mandatory to indicate in the supplemental box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

Box No. VII			INTERNATIONAL SEARCHING AUTHORITY		
Choice of International Searching Authority (ISA) (If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):			Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):		
			Date (day/month/year)	Number	Country (or regional Office)
ISA /					

Box No. VIII		CHECK LIST; LANGUAGE OF FILING	
This international application contains the following number of sheets		This international application is accompanied by the item(s) marked below:	
request	:5	1. <input checked="" type="checkbox"/> fee calculation sheet	
description (excluding sequence listing part)	:49	2. <input type="checkbox"/> separate signed power of attorney	
claims	:7	3. <input checked="" type="checkbox"/> copy of general power of attorney; reference number, if any:	
abstract	:1	4. <input type="checkbox"/> statement explaining lack of signature	
drawings	:15	5. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s):	
sequence listing part of description	:0	6. <input type="checkbox"/> translation of international application into (language):	
Total number of sheets	:77	7. <input type="checkbox"/> separate indications concerning deposited microorganisms or other biological matter	
		8. <input type="checkbox"/> nucleotide and/or amino acid sequence listing in computer readable form	
		9. <input checked="" type="checkbox"/> other (specify): 23/77 x 1	
Figure of the drawings which should accompany the abstract	0	Language of filing of the international application: ENGLISH	

Box No. IX		SIGNATURE OF APPLICANT OR AGENT	
Next to each signature indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).			
<p>.....</p> <p>KREMER, SIMON M.</p> <p>For WALTON, SEÁN M.</p> <p>APPOINTED AGENT</p>			

For receiving Office use only	
1. Date of actual receipt of the purported international application:	2. Drawings: <input type="checkbox"/> received: <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA/	
6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid	

For International Bureau use only	
Date of receipt of the record copy by the International Bureau:	

PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

WALTON, Sean, M
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

- 8 DEC 2000

Date of mailing (day/month/year) 21 November 2000 (21.11.00)	
Applicant's or agent's file reference SMW/EP5884200	IMPORTANT NOTIFICATION
International application No. PCT/GB00/03800	International filing date (day/month/year) 04 October 2000 (04.10.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 04 October 1999 (04.10.99)
Applicant ISIS INNOVATION LIMITED et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

<u>Priority date</u>	<u>Priority application No.</u>	<u>Country or regional Office or PCT receiving Office</u>	<u>Date of receipt of priority document</u>
04 Octo 1999 (04.10.99)	9923423.9	GB	13 Nove 2000 (13.11.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Magda BOUACHA

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

WALTON, Sean, M.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

Date of mailing (day/month/year) 12 April 2001 (12.04.01)		IMPORTANT NOTICE	
Applicant's or agent's file reference SMW/BP5884200			
International application No. PCT/GB00/03800	International filing date (day/month/year) 04 October 2000 (04.10.00)	Priority date (day/month/year) 04 October 1999 (04.10.99)	
Applicant ISIS INNOVATION LIMITED et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
AU,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE,AG,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EA,EE,EP,ES,
FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,
MN,MW,MX,MZ,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,
The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
12 April 2001 (12.04.01) under No. WO 01/25461

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

UTROPHIN GENE PROMOTER

The present invention is based on cloning of a genomic promoter region of the human utrophin gene and of the mouse utrophin gene.

5 The severe muscle wasting disorders Duchenne muscular dystrophy (DMD) and the less debilitating Becker muscular dystrophy (BMD) are due to mutations in the dystrophin gene resulting in a lack of dystrophin or abnormal expression of truncated forms of dystrophin, respectively. Dystrophin is a
10 large cytoskeletal protein (427kDa with a length of 125nm) which in muscle is located at the cytoplasmic surface of the sarcolemma, the neuromuscular junction (NMJ) and myotendinous junction (MTJ). It binds to a complex of proteins and glycoproteins spanning the sarcolemma called the dystrophin
15 associated glycoprotein complex (DGC). The breakdown of the integrity of this complex due to loss of, or impairment of dystrophin function, leads to muscle degeneration and the DMD phenotype.

The dystrophin gene is the largest gene so far identified in
20 man, covering over 2.7 megabases and containing 79 exons. The corresponding 14kb dystrophin mRNA is expressed predominantly in skeletal, cardiac and smooth muscle with lower levels in brain. Transcription of dystrophin in different tissues is regulated from either the brain promoter (predominantly active
25 in neuronal cells) or muscle promoter (differentiated myogenic cells, and primary glial cells) giving rise to differing first exons. A third promoter between the muscle promoter and the second exon of dystrophin regulates expression in cerebellar Purkinje neurons. Recently reviewed in (Tinsley, et al (1994)
30 *Proc Natl Acad Sci U S A* 91, 8307-13, Blake, et al (1994) *Trends in Cell Biol.* 4: 19-23, Tinsley, et al (1993) *Curr Opin Genet Dev.* 3: 484-90).

There are various approaches which have been adopted for the gene therapy of DMD, using the *mdx* mouse as a model system. However, there are considerable problems related to the number of muscle cells that can be made dystrophin positive, the
5 levels of expression of the gene and the duration of expression (Partridge, et al. (1995) *British Medical Bulletin* 51: 123-137). It has also become apparent that simply re-introducing genes expressing the dystrophin carboxy-terminus has no effect on the dystrophic phenotype although the DGC
10 appears to be re-established at the sarcolemma (Cox, et al. (1994) *Nature Genet* 8: 333-339, Greenberg, et al. (1994) *Nature Genet* 8: 340-344).

In order to circumvent some of these problems, possibilities of compensating for dystrophin loss using a related protein, utrophin, are being explored as an alternative route to
15 dystrophin gene therapy. A similar strategy is currently being evaluated in clinical trials to up-regulate foetal haemoglobin to compensate for the affected adult-globin chains in patients with sickle cell anaemia (Rodgers, et al. (1993) *N Engl J Med.* 328: 73-80, Perrine, et al. (1993) *N Engl J Med.* 328: 81-86).

Utrophin is a 395kDa protein encoded by multiexonic 1Mb *UTRN* gene located on chromosome 6q24 (Pearce, et al. (1993) *Hum Mol Gene.* 2: 1765-1772). At present the tissue regulation of
25 utrophin is not fully understood. In the dystrophin deficient *mdx* mouse, utrophin levels in muscle remain elevated soon after birth compared with normal mice; once the utrophin levels have decreased to the adult levels (about 1 week after birth), the first signs of muscle fibre necrosis are detected.
30 However there is evidence to suggest that in the small calibre muscles, continual increased levels of utrophin can interact with the DGC complex (or an antigenically related complex) at the sarcolemma thus preventing loss of the complex with the result that these muscles appear normal. There is also a

substantial body of evidence demonstrating that utrophin is capable of localising to the sarcolemma in normal muscle. During fetal muscle development there is increased utrophin expression, localised to the sarcolemma, up until 18 weeks in the human and 20 days gestation in the mouse. After this time the utrophin sarcolemmal staining steadily decreases to the significantly lower adult levels shortly before birth where utrophin is localised almost exclusively to the NMJ. The decrease in utrophin expression coincides with increased expression of dystrophin. See reviews (Ibraghimov Beskrovnaya, et al. (1992) *Nature* 355, 696-702., Blake, et al. (1994) *Trends in Cell Biol.* 4: 19-23, Tinsley, et al. (1993) *Curr Opin Genet Dev.* 3: 484-90).

Thus, in certain circumstances utrophin can localise to the sarcolemma probably at the same binding sites as dystrophin, through interactions with actin and the DGC. Accordingly, if expression of utrophin is sufficiently elevated, it may maintain the DGC and thus alleviate muscle degeneration in DMD/BMD patients (Tinsley, et al. (1993) *Neuromuscul Disord* 3, 537-9.).

However, manipulation of utrophin expression and screening for molecules able to upregulate expression is hampered by the limited understanding of utrophin expression regulation and its promoters. We have previously isolated a promoter element lying within the CpG island at the 5' end of the utrophin locus that is active in a broad range of cell types and tissues, and shown it to be synaptically regulated in vivo (Dennis, et al. (1996) *Nucleic Acids Res* 24, 1646-52 and WO 96/34101). The sequence contains a consensus N-box, a 6bp motif important in the regulation of other genes expressed at the NMJ (Koike, et al. (1995) *Proc Natl Acad Sci USA* 92, 10624-10628). Localisation of utrophin at the NMJ in mature muscle is partially attributable to enhanced transcription of utrophin at sub-junctional myonuclei, with consequent synaptic

accumulation of mRNA (Gramolini, et al. (1997) *J Biol Chem* 272, 8117-20, Vater, et al. (1998) *Molecular and Cellular Neuroscience* 10, 229-242). The utrophin promoter drives synaptic transcription of a reporter gene in vivo; this
5 expression pattern is abolished by point mutations within the N-box (Gramolin, et al. (1998) *J Biol Chem* 273, 736-43).

The present inventors hypothesised that utrophin might be transcribed from more than one promoter, an important consideration for the following reasons: First, it may be
10 undesirable to interfere with the mechanisms underlying synaptic regulation of genes, as this might affect expression of other post-synaptic components and impair the structure and function of the NMJ; a promoter without synaptic regulatory elements might be a more suitable target for pharmacological
15 manipulation. Second, cardiac dysfunction is a common feature of the dystrophinopathies (Hoogerwaard, et al. (1997) *J Neurol* 244, 657-63, Sasaki, et al. (1998) *Am Heart J* 135, 937-44); if the cardiac utrophin message was transcribed from a different promoter, then it might prove necessary to up-regulate this.
20 Finally, inclusion of additional regulatory sequences might increase the yield of a screening program to identify small molecules capable of transcriptional activation of utrophin.

We have now identified an alternative promoter lying within the large second intron of the utrophin gene, 50kb 3' to exon
25 2. The promoter is highly regulated, expressed in a wide range of tissues and has little similarity to the synaptically expressed promoter. This promoter drives transcription of a widely expressed unique first exon that splices into a common full-length mRNA at exon 3. This unique exon (called exon IB)
30 encodes a novel 31 amino acid N-terminus for the utrophin protein which may be involved in binding to the muscle membrane. The sequences of the two utrophin promoters are dissimilar, and we predict that they respond to discrete sets of cellular signals.

Exon IB is primarily considered herein to encode the indicated 31 amino acids. However, the splice occurs within a codon for aspartate. This aspartate residue is common to both isoforms of utrophin. In embodiments of the invention an aspartate residue may be included C-terminal to the 31 amino acids to provide a 32 amino acid peptide, which may be joined to additional amino acids, for instance additional utrophin sequence as discussed. See, for instance, Figure 8 for one embodiment.

10 These findings significantly contribute to the understanding of the molecular physiology of utrophin expression and are important because the promoter reported here provides an alternative target for transcriptional activation of utrophin in DMD muscle. This promoter does not contain synaptic
15 regulatory elements and might, therefore, be a more suitable target for pharmacological manipulation than the previously described promoter.

We have now cloned this alternative utrophin promoter and exon, and the present invention in various aspects and
20 embodiments is based on the sequence information obtained and provided herein.

One major use of the promoter is in screening for substances able to modulate its activity. It is well known that pharmaceutical research leading to the identification of a new
25 drug generally involves the screening of very large numbers of candidate substances, both before and even after a lead compound has been found. This is one factor which makes pharmaceutical research very expensive and time-consuming. A method or means assisting in the screening process will have
30 considerable commercial importance and utility. Substances identified as upregulators of the utrophin promoter represent an advance in the fight against muscular dystrophy since they provide basis for design and investigation of therapeutics for

in vivo use.

In one aspect, the present invention provides an isolated nucleic acid comprising a promoter, the promoter comprising a sequence of nucleotides shown in Figure 1 or Figure 2. The promoter may comprise one or more fragments of the sequence shown in Figure 1 or Figure 2 sufficient to promote gene expression. The promoter may comprise or consist essentially of a sequence of nucleotides 5' to position 1440 in Figure 1 (human) or position 1183 in Figure 2 (mouse). Preferably the promoter comprises or consists essentially of nucleotides 1199 to 1440 of the human sequence shown in Figure 1, or the equivalent sequence in mouse, e.g. nucleotides 959 to 1183 of Figure 2.

An even smaller portion of this part of the sequences shown in Figure 1 or Figure 2 may be used as long as promoter activity is retained. Restriction enzymes or nucleases may be used to digest the nucleic acid, followed by an appropriate assay (for example as illustrated herein using luciferase constructs) to determine the minimal sequence required. A preferred embodiment of the present invention provides a nucleic acid isolate with the minimal nucleotide sequence shown in Figure 1 or Figure 2 required for promoter activity. The minimal promoter element is situated between the PvuII restriction site at position 1199 in the human sequence and the transcription start site at 1440 bp in the human sequence and between nucleotides 959 to 1183 in the mouse sequence (see Figure 2).

In one embodiment a promoter according to the present invention comprises or consists of sequence that is shown in Figure 3 to be conserved between the human and mouse sequences, e.g. the 25 nucleotide sequence:
ACAGGACATCCCAGTGTGCAGTTCG spanning the transcriptional start site.

The promoter may comprise one or more sequence motifs or elements conferring developmental and/or tissue-specific regulatory control of expression. For instance, the promoter may comprise a sequence for muscle-specific expression, e.g.
5 an E-box element/myoD binding site, such as CANNTG, preferably CAGGTG.

Other regulatory sequences may be included, for instance as identified by mutation or digest assay in an appropriate expression system or by sequence comparison with available
10 information, e.g. using a computer to search on-line databases.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of
15 double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation
20 regulation" of the promoter.

The present invention extends to a promoter which has a nucleotide sequence which is allele, mutant, variant or derivative, by way of nucleotide addition, insertion, substitution or deletion of a promoter sequence as provided
25 herein. Systematic or random mutagenesis of nucleic acid to make an alteration to the nucleotide sequence may be performed using any technique known to those skilled in the art. One or more alterations to a promoter sequence according to the present invention may increase or decrease promoter activity,
30 or increase or decrease the magnitude of the effect of a substance able to modulate the promoter activity.

"Promoter activity" is used to refer to ability to initiate transcription. The level of promoter activity is quantifiable for instance by assessment of the amount of mRNA produced by transcription from the promoter or by assessment of the amount of protein product produced by translation of mRNA produced by transcription from the promoter. The amount of a specific mRNA present in an expression system may be determined for example using specific oligonucleotides which are able to hybridise with the mRNA and which are labelled or may be used in a specific amplification reaction such as the polymerase chain reaction. Use of a reporter gene as discussed further below facilitates determination of promoter activity by reference to protein production.

In various embodiments of the present invention a promoter which has a sequence that is a fragment, mutant, allele, derivative or variant, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the sequence of either the human or the mouse promoters shown in Figures 1 and 2, respectively, has at least about 60% homology with one or both of the shown sequences, preferably at least about 70% homology, more preferably at least about 80% homology, more preferably at least about 90% homology, more preferably at least about 95% homology. The sequence in accordance with an embodiment of the invention may hybridise with one or both of the shown sequences, or the complementary sequences (since DNA is generally double-stranded).

Similarity or homology (the terms are used interchangeably) or identity is preferably determined using GAP, from version 20 of GCG. This uses the algorithm of Needleman and Wunsch to align sequences inserting gaps as appropriate to improve the agreement between the two sequences. Parameters employed are the default ones: for nucleotide sequences - Gap Weight 50, Length Weight 3, Average Match 10.000, Average Mismatch 0.000; for peptide sequences - Gap Weight 8, Length Weight 2, Average

Match 2.912, Average Mismatch -2.003. Peptide similarity scores are taken from the BLOSUM62 matrix. Also useful is the TBLASTN program, of Altschul et al. (1990) *J. Mol. Biol.* 215: 403-10, or BestFit, which is part of the Wisconsin Package, 5 Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). Sequence comparisons may be made using FASTA and FASTP (see Pearson & Lipman, 1988. *Methods in Enzymology* 183: 63-98). Parameters are preferably set, using the default matrix, as 10 follows: Gapopen (penalty for the first residue in a gap): -12 for proteins / -16 for DNA; Gapext (penalty for additional residues in a gap): -2 for proteins / -4 for DNA; KTUP word length: 2 for proteins / 6 for DNA.

15 Nucleic acid sequence homology may be determined by means of selective hybridisation between molecules under stringent conditions.

Preliminary experiments may be performed by hybridising under low stringency conditions. For probing, preferred conditions are those which are stringent enough for there to be a simple 20 pattern with a small number of hybridisations identified as positive which can be investigated further.

For example, hybridizations may be performed, according to the method of Sambrook et al. (below) using a hybridization solution comprising: 5X SSC (wherein 'SSC' = 0.15 M sodium 25 chloride; 0.15 M sodium citrate; pH 7), 5X Denhardt's reagent, 0.5-1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as 30 follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes - 1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65°C in 1X SSC and 1% SDS, changing the solution

every 30 minutes.

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology is (Sambrook et al., 1989): $T_m = 81.5^{\circ}\text{C} + 16.6\text{Log} [\text{Na}^+] + 0.41 (\% \text{ G+C}) - 0.63 (\% \text{ formamide}) - 600/\text{\#bp in duplex}.$

As an illustration of the above formula, using $[\text{Na}^+] = [0.368]$ and 50-% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C . The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C . Such a sequence would be considered substantially homologous to the nucleic acid sequence of the present invention.

It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain. Other suitable conditions include, e.g. for detection of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na_2HPO_4 , pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na_2HPO_4 , pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

In a further embodiment, hybridisation of nucleic acid molecule to an allele or variant may be determined or identified indirectly, e.g. using a nucleic acid amplification reaction, particularly the polymerase chain reaction (PCR). PCR requires the use of two primers to specifically amplify target nucleic acid, so preferably two nucleic acid molecules

with sequences characteristic of the utrophin promoter are employed. Using RACE PCR, only one such primer may be needed (see "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, Academic Press, New York, (1990)).

5 Thus a method involving use of PCR in obtaining nucleic acid according to the present invention may include:

(a) providing a preparation of nucleic acid, e.g. from a muscle cell;

10 (b) providing a pair of nucleic acid molecule primers useful in (i.e. suitable for) PCR, at least one of said primers being a primer specific for nucleic acid according to the present invention;

(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR;

15 (d) performing PCR and determining the presence or absence of an amplified PCR product.

The presence of an amplified PCR product may indicate identification of an allele or other variant. The sequence may have the ability to promote transcription (i.e. have
20 "promoter activity") in muscle cells, e.g. human muscle cells, or muscle-specific transcription.

Further provided by the present invention is a nucleic acid construct comprising a utrophin promoter region or a fragment, mutant, allele, derivative or variant thereof able to promoter
25 transcription, operably linked to a heterologous gene, e.g. a coding sequence. By "heterologous" is meant a gene other than utrophin. Modified forms of utrophin are generally excluded. Generally, the gene may be transcribed into mRNA which may be translated into a peptide or polypeptide product which may be
30 detected and preferably quantitated following expression. A gene whose encoded product may be assayed following expression is termed a "reporter gene", i.e. a gene which "reports" on promoter activity.

The reporter gene preferably encodes an enzyme which catalyses a reaction which produces a detectable signal, preferably a visually detectable signal, such as a coloured product. Many examples are known, including β -galactosidase and luciferase.

5 β -galactosidase activity may be assayed by production of blue colour on substrate, the assay being by eye or by use of a spectrophotometer to measure absorbance. Fluorescence, for example that produced as a result of luciferase activity, may be quantitated using a spectrophotometer. Radioactive assays

10 may be used, for instance using chloramphenicol acetyltransferase, which may also be used in non-radioactive assays. The presence and/or amount of gene product resulting from expression from the reporter gene may be determined using a molecule able to bind the product, such as an antibody or

15 fragment thereof. The binding molecule may be labelled directly or indirectly using any standard technique.

Those skilled in the art are well aware of a multitude of possible reporter genes and assay techniques which may be used to determine gene activity. Any suitable reporter/assay may

20 be used and it should be appreciated that no particular choice is essential to or a limitation of the present invention.

Expression of a reporter gene from the promoter may be in an *in vitro* expression system or may be intracellular (*in vivo*). Expression generally requires the presence, in addition to the

25 promoter which initiates transcription, a translational initiation region and transcriptional and translational termination regions. One or more introns may be present in the gene, along with mRNA processing signals (e.g. splice sites).

30 Systems for cloning and expression of a polypeptide are discussed further below.

The present invention also provides a nucleic acid vector

comprising a promoter as disclosed herein. Such a vector may comprise a suitably positioned restriction site or other means for insertion into the vector of a sequence heterologous to the promoter to be operably linked thereto.

5 Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular*
10 *Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Procedures for introducing DNA into cells depend on the host used, but are well known.

Thus, a further aspect of the present invention provides a
15 host cell containing a nucleic acid construct comprising a promoter element, as disclosed herein, operably linked to a heterologous gene. A still further aspect provides a method comprising introducing such a construct into a host cell. The introduction may employ any available technique, including,
20 for eukaryotic cells, calcium phosphate transfection, DEAE-Dextran transfection, electroporation, liposome-mediated transfection and transduction using retrovirus.

The introduction may be followed by causing or allowing expression of the heterologous gene under the control of the
25 promoter, e.g. by culturing host cells under conditions for expression of the gene.

In one embodiment, the construct comprising promoter and gene is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion in the
30 construct of sequences which promote recombination with the genome, in accordance with standard techniques.

Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are
5 described in detail in *Current Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1994, the disclosure of which is incorporated herein by reference.

Nucleic acid molecules, constructs and vectors according to the present invention may be provided isolated and/or purified
10 (i.e. from their natural environment), in substantially pure or homogeneous form, free or substantially free of a utrophin coding sequence, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the promoter sequence. Nucleic acid according to the present
15 invention may be wholly or partially synthetic. The term "isolate" encompasses all these possibilities.

Nucleic acid constructs comprising a promoter (as disclosed herein) and a heterologous gene (reporter) may be employed in screening for a substance able to modulate utrophin promoter
20 activity. For therapeutic purposes, e.g. for treatment of muscular dystrophy, a substance able to up-regulate expression of the promoter may be sought. A method of screening for ability of a substance to modulate activity of a utrophin promoter may comprise contacting an expression system, such as
25 a host cell, containing a nucleic acid construct as herein disclosed with a test or candidate substance and determining expression of the heterologous gene. The level of transcription of the heterologous gene, or the level of heterologous protein may be determined. The level of protein
30 may be determined by measuring the amount of protein, or the activity of the protein, using techniques known to those skilled in the art.

Alternatively, or additionally a method of screening for

ability of a substance to modulate activity of a utrophin promoter may comprise contacting a cell containing an endogenous utrophin gene (e.g. a mammalian muscle cell) with a test substance and measuring the level of RNA transcription or protein expression using binding members specific for the nucleic acid or polypeptides disclosed herein. Specific binding members include antibodies and nucleic acid probes.

The level of expression in the presence of the test substance may be compared with the level of expression in the absence of the test substance. A difference in expression in the presence of the test substance indicates ability of the substance to modulate gene expression. An increase in expression of the heterologous gene compared with expression of another gene not linked to a promoter as disclosed herein indicates specificity of the substance for modulation of the utrophin promoter.

A promoter construct may be transfected into a cell line using any technique previously described to produce a stable cell line containing the reporter construct integrated into the genome. The cells may be grown and incubated with test compounds for varying times. The cells may be grown in 96 well plates to facilitate the analysis of large numbers of compounds. The cells may then be washed and the reporter gene expression analysed. For some reporters, such as luciferase, the cells will be lysed then analysed. Previous experiments testing the effects of glucocorticoids on the endogenous utrophin protein and RNA levels in myoblasts have already been described [12,13] and techniques used for those experiments may similarly be employed.

Constructs comprising one or more developmental and/or time-specific regulatory motifs (as discussed) may be used to screen for a substance able to modulate the corresponding aspect of the promoter activity, e.g. muscle-specific

expression.

Following identification of a substance which modulates or affects utrophin promoter activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

As noted above, the inventors also identified a novel coding sequence (Exon IB) which encodes a novel utrophin N-terminus.

According to a further aspect of the present invention there is provided a nucleic acid molecule which has a nucleotide sequence encoding a polypeptide which includes the amino acid sequence shown in Figure 1 or Figure 2. Such a polypeptide may include other utrophin sequences, and the nucleic acid molecule may be in the form of a utrophin "mini-gene" (discussed further below).

Such a polypeptide may include non-utrophin (i.e. heterologous or foreign) sequences and thereby form a larger fusion protein. For example, such a fusion protein could be used to target a non-utrophin polypeptide to muscle membranes.

The coding sequence included may be that shown in Figure 1 or Figure 2 or it may be a mutant, variant, derivative or allele of the sequence shown. The sequence may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may

include a sequence different from the sequences shown in Figure 1 or Figure 2 yet encode a polypeptide with the same amino acid sequence. The amino acid sequences shown in Figure 1 and figure 2 consist of 31 residues.

5 On the other hand the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequences shown in Figure 1 or Figure 2. Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant, derivative or allele of
10 the sequences shown in Figure 1 and Figure 2 are further provided by the present invention. Nucleic acid encoding such a polypeptide may show at the nucleotide sequence and/or encoded amino acid level greater than about 60% homology with the coding sequence and/or the amino acid sequence shown in
15 Figure 1 or Figure 2, greater than about 70% homology, greater than about 80% homology, greater than about 90% homology or greater than about 95% homology. Determination of homology is discussed elsewhere herein.

A polypeptide which is a variant, allele, derivative or mutant
20 may have an amino acid sequence which differs from that given in a figure herein by one or more of addition, substitution, deletion and insertion of one or more amino acids. Preferred such polypeptides have wild-type function, that is to say have one or more of the following properties: immunological cross-
25 reactivity with an antibody reactive the polypeptide for which the sequence is given in Figure 1 or Figure 2; sharing an epitope with the polypeptide for which the amino acid sequence is shown in Figure 1 or Figure 2 (as determined for example by immunological cross-reactivity between the two polypeptides);
30 a biological activity which is inhibited by an antibody raised against the polypeptide whose sequence is shown in Figure 1 or Figure 2; ability to bind muscle membrane, ability to bind actin; ability to bind DPC.

Variations in amino acid sequence include "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as
5 arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Particular amino acid sequence variants may differ from that shown in Figure 1 or Figure 2 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, or 5-10 amino acids.

10 According to one aspect of the present invention there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding a polypeptide with utrophin function. Utrophin nucleotide sequences which may be included in the nucleic acid molecule are disclosed in WO 97/922696 which is
15 incorporated herein by reference.

See also Figure 8 and Figure 9 for disclosure of nucleic acid molecules and polypeptides according to the present invention, comprising the exon IB sequence of the invention.

A polypeptide with utrophin function is able to bind actin and
20 able to bind the dystrophin protein complex (DPC).

The nucleic acid molecule may be an isolate, or in an isolated and/or purified form, that is to say not in an environment in which it is found in nature, removed from its natural environment. It may be free from other nucleic acid
25 obtainable from the same species, e.g. encoding another polypeptide.

In one embodiment, nucleic acid molecule is a "mini-gene", i.e. the polypeptide encoded does not correspond to full-length utrophin but is rather shorter, a truncated version
30 (Utrophin mini-genes are discussed in WO97/22696). For instance, part or all of the rod domain may be missing, such

that the polypeptide comprises an actin-binding domain and a DPC-binding domain but is shorter than naturally occurring utrophin. In a full-length utrophin gene including what are identified herein as exons 1A and 1B, the actin-binding domain is encoded by nucleotides 1-739, while the DPC-binding domain (CRCT) is encoded by nucleotides 8499-10301 (where 1 represents the start of translation). See also Figure 8. The respective domains in the polypeptide encoded by a mini-gene according to the invention may comprise amino acids corresponding to those encoded by these nucleotides in the full-length coding sequence. In one embodiment, a minigene according to the present invention comprises or consists of the amino acid sequence encoded by nucleotides 1-739 and 8499-10301 of the A isoform of utrophin in which exon 1B as identified herein is substituted for exons 1A and 2A. The sequence of such a minigene can be constructed by the ordinary skilled person using information disclosed herein, taking into account the content of WO97/22696 and Tinsley et al, *Nature* (1996) 384:349. The nucleic acid sequence and predicted amino acid sequence encoded by a 'mini-gene' according to the present invention are shown in Figure 9.

Advantages of a mini-gene over a sequence encoding a full-length utrophin molecule or derivative thereof include easier manipulation and inclusion in vectors, such as adenoviral and retroviral vectors for delivery and expression.

A further preferred non-naturally occurring nucleic acid molecule encoding a polypeptide with the specified characteristics is a chimaeric construct wherein the encoding sequence comprises a sequence obtainable from one mammal, preferably human ("a human sequence"), and a sequence obtainable from another mammal, preferably mouse ("a mouse sequence"). Such a chimaeric construct may of course comprise the addition, insertion, substitution and/or deletion of one or more nucleotides with respect to the parent mammalian

sequences from which it is derived. Preferably, the part of the coding sequence which encodes the actin-binding domain comprises a sequence of nucleotides obtainable from the mouse, or other non-human mammal, or a sequence of nucleotides
5 derived from a sequence obtainable from the mouse, or other non-human mammal.

In a preferred embodiment, the sequence of nucleotides encoding the polypeptide comprises sequence GAGGCAC at residues 331-337 and/or the sequence GATTGTGGATGAAAACAGTGGG at
10 residues 1453-1475 (using the conventional numbering from the initiation codon ATG), and a sequence obtainable from a human.

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridise with one or more fragments of a nucleic acid
15 sequence shown in Figure 1 or Figure 2 particularly fragments of relatively rare sequence, based on codon usage or statistical analysis. The amino acid sequence information provided may be used in design of degenerate probes/primers or "long" probes. A primer designed to hybridise with a fragment
20 of the nucleic acid sequence shown may be used in conjunction with one or more oligonucleotides designed to hybridise to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE" (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an
25 oligonucleotide linker and PCR is performed using a primer which hybridises with the sequence shown in the figures and a primer which hybridises to the oligonucleotide linker.

Nucleic acid isolated and/or purified from one or more cells (e.g. human, mouse) or a nucleic acid library derived from
30 nucleic acid isolated and/or purified from cells (e.g. a cDNA library derived from mRNA isolated from the cells), may be probed under conditions for selective hybridisation and/or subjected to a specific nucleic acid amplification reaction

such as the polymerase chain reaction (PCR).

A method may include hybridisation of one or more (e.g. two) probes or primers to target nucleic acid. Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. A screening procedure, chosen from the many available to those skilled in the art, is used to identify successful hybridisation events and isolated hybridised nucleic acid.

Probing may employ the standard Southern blotting technique. For instance DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells.

Preliminary experiments may be performed by hybridising under low stringency conditions various probes to Southern blots of DNA digested with restriction enzymes. Suitable conditions would be achieved when a large number of hybridising fragments were obtained while the background hybridisation was low. Using these conditions nucleic acid libraries, e.g. cDNA libraries representative of expressed sequences, may be searched.

It may be necessary for one or more gene fragments to be ligated to generate a full-length coding sequence. Also, where a full-length encoding nucleic acid molecule has not been obtained, a smaller molecule representing part of the

full molecule, may be used to obtain full-length clones. Inserts may be prepared from partial cDNA clones and used to screen cDNA libraries.

Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on. Exemplary conditions have been discussed already above.

5 Nucleic acid according to the present invention may form part of a cloning vector and/or a vector from which the encoded polypeptide may be expressed. Polypeptide expression is discussed below. Suitable vectors can be chosen or constructed, containing appropriate and appropriately positioned regulatory sequences, as discussed elsewhere
15 herein.

A further aspect of the present invention provides a polypeptide which comprises the amino acid sequence shown in Figure 1 or Figure 2. As mentioned earlier such a polypeptide
20 may include other utrophin sequences or may include heterologous sequences.

Polypeptides which are amino acid sequence variants, alleles, derivatives or mutants are also provided by the present invention. Such polypeptides are discussed elsewhere herein.

25 The skilled person can use the techniques described herein and others well known in the art to produce large amounts of peptides, for instance by expression from encoding nucleic acid.

In a further aspect the invention provides a method of making
30 a polypeptide, the method including expression from nucleic

acid encoding the polypeptide (generally nucleic acid according to the invention). This may be conveniently be achieved by growing in culture a host cell containing such a vector, under suitable conditions which cause or allow
5 expression of the polypeptide. Polypeptides may also be expressed in in vitro systems such as reticulocyte lysate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus
10 systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. A common, preferred bacterial host is *E. coli*.

Thus, a further aspect of the present invention provides a
15 host cell containing heterologous nucleic acid encoding a polypeptide as disclosed herein.

The nucleic acid may be integrated into the genome (e.g. chromosome) of the host cell or may be on an extra-chromosomal vector within the cell, or otherwise identifiably heterologous
20 or foreign to the cell.

A still further aspect provides a method comprising introducing such nucleic acid into a host cell. Suitable techniques are discussed elsewhere herein.

The introduction may be followed by causing or allowing
25 expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

The polypeptide encoded by the nucleic acid may be expressed from the nucleic acid *in vitro*, e.g. in a cell-free system or in cultured cells, or *in vivo*.

30 If the polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into

the culture medium.

Peptides can also be generated wholly or partly by chemical synthesis. The compounds of the present invention can be readily prepared according to well-established, standard
5 liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The
10 Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first
15 completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

20 The present invention also includes active portions, fragments, derivatives and functional mimetics of the polypeptides of the invention. An "active portion" of a polypeptide means a peptide which is less than said full length polypeptide, but which retains a biological activity,
25 such as a biological activity selected from binding to ligand, binding to muscle membrane. Such an active fragment may be included as part of a fusion protein, e.g. including a polypeptide which is to be targetted to the muscle membrane.

A "fragment" of a polypeptide generally means a stretch of
30 amino acid residues of about five to twenty-five contiguous amino acids, typically about ten to twenty contiguous amino acids. Fragments of the novel N-terminus polypeptide sequence may include antigenic determinants or epitopes useful for

raising antibodies to a portion of the amino acid sequence, or may be sequence useful for targetting to muscle membrane. Alanine scans are commonly used to find and refine peptide motifs within polypeptides, this involving the systematic
5 replacement of each residue in turn with the amino acid alanine, followed by an assessment of biological activity.

Preferred fragments of exon 1B polypeptide include those comprising or consisting of an epitope which may be used for instance in raising or isolating antibodies. Variant and
10 derivative peptides, peptides which have an amino acid sequence which differs from one of these sequences by way of addition, insertion, deletion or substitution of one or more amino acids are also provided by the present invention.

A "derivative" of a polypeptide or a fragment thereof may
15 include a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve one or more of insertion, addition, deletion or
20 substitution of one or more amino acids, which may be without fundamentally altering the qualitative nature of biological activity of the wild type polypeptide. Also encompassed within the scope of the present invention are functional mimetics of active fragments of the exon 1B polypeptides
25 provided (including alleles, mutants, derivatives and variants). The term "functional mimetic" means a substance which may not contain an active portion of the relevant amino acid sequence, and probably is not a peptide at all, but which retains in qualitative terms biological activity of natural
30 exon 1B polypeptide. The design and screening of candidate mimetics is described in detail below.

A polypeptide according to the present invention may be isolated and/or purified (e.g. using an antibody) for instance

after production by expression from encoding nucleic acid (for which see below). Thus, a polypeptide may be provided free or substantially free from contaminants with which it is naturally associated (if it is a naturally-occurring polypeptide). A polypeptide may be provided free or substantially free of other polypeptides. Polypeptides according to the present invention may be generated wholly or partly by chemical synthesis. The isolated and/or purified polypeptide may be used in formulation of a composition, which may include at least one additional component, for example a pharmaceutical composition including a pharmaceutically acceptable excipient, vehicle or carrier. A composition including a polypeptide according to the invention may be used in prophylactic and/or therapeutic treatment as discussed below.

A polypeptide, peptide, allele, mutant, derivative or variant according to the present invention may be used as an immunogen or otherwise in obtaining specific antibodies. Antibodies are useful in purification and other manipulation of polypeptides and peptides, diagnostic screening and therapeutic contexts.

Accordingly, a further aspect of the present invention provides an antibody able to bind specifically to the polypeptide whose sequence is given in Figure 1 or Figure 2. Such an antibody may be specific in the sense of being able to distinguish between the polypeptide it is able to bind and other human (or mouse) polypeptides for which it has no or substantially no binding affinity (e.g. a binding affinity of about 1000x less). Specific antibodies bind an epitope on the molecule which is either new present or is not accessible on other molecules. Antibodies according to the present invention may be specific for the wild-type polypeptide. Antibodies according to the invention may be specific for a particular mutant, variant, allele or derivative polypeptide as between that molecule and the wild-type polypeptide, so as

to be useful in diagnostic and prognostic methods as discussed below. Antibodies are also useful in purifying the polypeptide or polypeptides to which they bind, e.g. following production by recombinant expression from encoding nucleic acid.

5 Preferred antibodies according to the invention are isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention.

10 Antibodies may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. 15 Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques (Armitage et al., 1992, 20 immunoprecipitation may be used (Armitage et al., 1992, Nature 357: 80-82). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

25 As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; 30 for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained

from an organism which has been exposed to the antigen of interest.

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be
5 construed as covering any binding substance having a binding domain with the required specificity. Thus the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic molecules and molecules whose shape mimicks that of an antibody enabling
10 it to bind an antigen or epitope.

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, C1 and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and
15 VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')₂ fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

20 A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other changes. It will further be understood by those skilled in the art that a monoclonal antibody can be subjected to the techniques of recombinant DNA technology to produce other
25 antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus
30 framework regions, of a different immunoglobulin. See, for instance, EP184187A, GB 2188638A or EP-A-0239400. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

- 10 The reactivities of antibodies on a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be
15 directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody with
20 an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

- 25 Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or
30 otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions

between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems
5 may be employed.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge. Particular embodiments of antibodies
10 according to the present invention include antibodies able to bind and/or which bind specifically, e.g. with an affinity of at least 10^{-7} M, to the peptides shown in Figure 1 or Figure 2.

Antibodies according to the present invention may be used in screening for the presence of a polypeptide, for example in a
15 test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid therefor.

20 An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling molecules, buffer solutions, elutants and so on. Reagents may
25 be provided within containers which protect them from the external environment, such as a sealed vial.

The present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of utrophin promoter activity, or to a polypeptide,
30 or nucleic acid molecule in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a

method comprising administration of such a composition to a patient, e.g. for increasing utrophin expression for instance in treatment of muscular dystrophy, use of such a substance in manufacture of a composition for administration, e.g. for
5 increasing utrophin expression for instance in treatment of muscular dystrophy, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

10 Administration will preferably be in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature
15 and severity of what is being treated. Prescription of treatment, eg decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

A composition may be administered alone or in combination with
20 other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a
25 pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on
30 the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

Pharmaceutical compositions for oral administration may be in

tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Instead of a substance identified using a promoter as disclosed herein, a mimetic or mimic or the substance may be designed for pharmaceutical use. The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, eg peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing may be used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property.

Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, eg by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled to according its physical properties, eg stereochemistry, bonding, size and/or charge, using data from a range of sources, eg spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process. In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Mimetics of substances identified as having ability to

modulate utrophin promoter activity using a screening method as disclosed herein are included within the scope of the present invention.

5 Modifications to and further aspects and embodiments of the present invention will be apparent to those skilled in the art. All documents mentioned herein are incorporated by reference.

10 Experimental basis for and embodiments of the present invention will now be described in more detail, by way of example and not limitation, and with reference to the following figures:

Figure 1 shows the sequence of the human exon 1B and promoter B. Numbering corresponds to the insert of pBSX2.0. The deduced translation of exon 1B is shown. The positions of features
15 such as restriction sites, IL-6 response element and Alu repetitive elements are shown.

Figure 2 shows the sequence of the mouse exon 1B and promoter B. Numbering corresponds to the insert of pBSX8.0. The deduced translation of exon 1B is shown. The positions of features
20 such as restriction sites, IL-6 response element and Alu repetitive elements are shown.

Figure 3 shows the sequence alignment of human (top) and mouse (bottom) exon 1B (in upper case) and promoter B. Numbering corresponds to the inserts of pBSX2.0 and pBSX8.0,
25 respectively. The human PvuII site (see Figure 7) is indicated. The open triangle indicates the position at which the luciferase coding sequence was inserted to make pGL3/UtroB/F (see below). The deduced translation of exon 1B is shown; amino acids marked in bold type are identical
30 between the human and mouse sequences. The conserved splice donor consensus is shown in grey. Two putative Ap1 sites and

an initiator-like element (Inr) are 100% conserved and indicated in black. A solid arrow marks the single transcription start indicated by primer extension; figures adjacent to the sequence indicate the number of individual
5 5'RACE clones that terminated at the positions shown.

Figure 4 shows the position of the primers used in RT-PCR of exon 1B-containing utrophin transcript, and the probes used to probe the PCR products. Primers specific to exon 1B (BF31) and utrophin C-terminus (CT2) were used to amplify 9816bp of
10 utrophin cDNA. The products were blotted and probed with U41, U107, BR4 and U16 as indicated. The diagram is not to scale; numbering refers to the nucleotide sequence of the full-length cDNA. The corresponding functional domains of the protein are indicated above: actin binding domain; rod, rod domain; Cys,
15 cysteine rich domain, C-Term; C-terminal domain.

Figure 5 shows a schematic representation of (A) human YAC and (B) mouse PAC contigs showing position of exons within the genomic map. Key to mouse restriction sites: C, ClaI; S,
20 SacII; B, BssHII; X, XhoI. (C) shows the nomenclature for utrophin promoters, exons and transcripts.

Figure 6 shows the *in vitro* activity of utrophin promoter B. (A) shows normalised luciferase activity following transfection of three different human cell types with either
25 pGL3/utroB/F ('forward construct') or pGL3/utroB/R ('reverse construct').

Figure 7 shows deletion analysis of promoter B. The 1.5kb insert of pGL3/utroB/F was deleted at its 5' and 3' ends using the internal restriction sites indicated. Reporter activity
30 was assayed following transient transfection of IN157 and CL11T47 cells.

Figure 8 shows conceptual translation of exon 1B as part of

utrophin, showing a nucleotide sequence and encoded polypeptide according to embodiments of the present invention.

Figure 9 shows the nucleic acid and predicted amino acid sequence of a utrophin B isoform 'minigene'.

- 5 Figure 10 shows the dosage dependence of IL-6 mediated expression from the isoform B promoter.

Oligonucleotides, PCR, RT-PCR and 5'RACE

PCR and RT-PCR were performed as described (Blake, et al. (1996) *J Biol Chem* 271, 7802-7810). Oligonucleotide sequences

10 (5' to 3') were:

	UM83	gatgttcctg tgaggccttc gag,
	UM82	cactcttgga aaatcgagcg t,
	U16	actatgatgt ctgccagagt tg,
	U107	gatccaatag cttccttcca tcttt,
15	UBF	tggaaaaagt ggagggttgga,
	BR2	tccaacctcc actttttcca,
	BR4	gcctggagag ctacatgcc t,
	BF8	ctccacatct ttttcctcat catct,
	BF9	gattgtggtg atggttgtag aa,
20	BR10	gattgtggtg atggttgtag aa,
	BR14	gatgatgagg aaaaagatgt ggag,
	BF15	aaacccaaaa taacacagga catc,
	BF16	agtgttaactt ctctctggtg,
	BF31	taagcagatg taggtgatga gc,
25	BF42	gctgcttttg ttgtccactt c,
	BR43	atagcttcct tccatctttg ag,
	CT2	ctccacgttc ttcctctct act,
	2ApF	gcgtgcagtg gaccattttt cagattta,
	1BpF	cgctgcagca gccaccacat ttcgttg,
30	3pR	gcgtgcagat cgagcgttta tccatttg.

5' RACE was undertaken using adapter-ligated mouse heart cDNA (Marathon-Ready, Clontech), following the manufacturer's

protocol, using the supplied adapter primers with nested mouse utrophin primers UM83 (exon 4) and UM82 (exon 3). Products were cloned in pGEM-T (Promega). Human exon 1B was isolated from skeletal muscle cDNA by PCR using mouse primers UBF and UM83. 5'RACE was used to clone the 5' end of human exon 1B, using primers U107 and BR4. Full-length utrophin RT-PCR was done as described (Blake, et al. (1996) *J Biol Chem* 271, 7802-7810.), but using Boehringer Expand Reverse Transcriptase and Long Template PCR reagents, and a primer annealing temperature of 59°C. Semi-quantitative RT-PCR was performed using primers BF42 and BR43 to amplify utrophin B, and commercial primers (Stratagene) to amplify glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Exponential amplification was established by withdrawing samples from thermal cycling at 1 cycle intervals over a range of 5 cycles, predicted to span the exponential range following initial experiments in which samples were withdrawn at 5 cycle intervals. Products were blotted and probed with labelled BR4 or a 600bp GA3PH probe. Band intensities were quantified using a Storm phosphorimager. A graph of \log_2 [band intensity] versus cycle number showed a linear relationship with gradient ≈ 1 , indicating near-perfect exponential amplification. The band intensities at any given cycle over this range are therefore directly proportional to the amount of cDNA in the original samples.

25 Genomic Mapping and Clones

Human YACs are as previously described (Pearce, et al. (1993) *Hum Mol Genet* 2, 1765-72). Southern blots of restriction digested YAC DNA were probed with end-labelled BR4. A 3.0kb hybridising XbaI fragment was cloned from YAC 4X124H10 (a YAC clone which contains a human genomic DNA insert) into pBlueScript (Stratagene) generating pBSX2.0. Mouse PACs were identified from the RPCI21 library. A 398bp exon 1B/promoter B DNA probe (UB400) encompassing human positions 1129 to 1527 was used for exon 1B mapping. Library filters were screened with probes to exons 1A-5 (Dennis, et al. (1996) *Nucleic Acid*

Res 24, 1646-52) and UB400. Eleven PACs were identified, and four of these arranged into a contig by restriction mapping. An 8.0kb XbaI fragment from PAC 110C24, that hybridised with UB400, was cloned in pBlueScript generating pBSX8.0.

5 Northern Blots and Probes

A human multiple tissue northern blot and b-actin control cDNA probe were obtained from Clontech. A utrophin C-terminal cDNA probe, encompassing the last 4.0kb of the utrophin message, was generated by PCR. Human exon 1B sequence between positions
10 1480 and 1596 was cloned into pGEM-T and an exon 1B antisense riboprobe was transcribed (In Vitro Transcription Kit, Promega) from the SP6 promoter following linearisation of the plasmid with NcoI. Hybridisation was carried out at 70°C in 50% formamide hybridisation buffer (Ausubel, et al. (1999)
15 *Current Protocols in Molecular Biology* (Wiley).) and the filter was washed at 75°C in 0.1xSSC, 0.1%SDS for 2 hours.

RNase Protection

Specific probes spanning the exon 1B/3 and exon 2A/3 boundaries were obtained by PCR amplification of mouse heart
20 cDNA using primers 2ApF, 1BpF and 3pR. Products were cloned in the PstI site of pDP18 (Ambion) and sequenced. Plasmids were linearised with EcoRI (1B) or BamHI (2A); labelled antisense riboprobe was transcribed from the T7 promoter and gel purified. RNase protection was carried out using RPAIII
25 kit (Ambion) following the manufacturer's instructions (30µg total RNA unless stated, hybridisation temperature 42°C, RNase A/T1 dilution 1:200). Following electrophoretic separation, band intensities were quantified as above, and corrected for the amount of label present in each protected fragment.

30 Promoter/Reporter Constructs

Reporter constructs were generated by PCR amplification of the human sequence between positions 39 and 1503, using pBSX2.0 as template. Pfu polymerase was used with primers BF9 and BR14.

Following 15 cycles of 96°C for 45 seconds, 62°C for 45 seconds, 72°C for 4 minutes, products were dA-tailed and cloned in pGEM-T. Clones were identified with product in both orientations and insert, liberated by digestion with
5 SacI/NcoI, was cloned into the SacI/NcoI sites of a promoterless luciferase reporter plasmid (pGL3 basic, Promega), generating constructs with insert in forward (pGL3/utroB/F) and reverse (pGL3/UtroB/R) orientation with respect to the coding sequence of luciferase. Deletions of the
10 forward construct were generated by cleavage at SpeI, NdeI, EcoRI and PvuII sites in the insert, followed by religation to sites in the 5' or 3' polylinker. Constructs were sequenced completely.

Cell Culture and Transfections

15 Three human cell lines (IN157 rhabdomyosarcoma (Nielsen et al., 1993, *Mol Cell Endocrinol* 93: 87-95), CL11T47 kidney epithelial and HeLa cervical epithelial (*Cancer Research*, 1952 12: 264) were maintained as described (Dennis, et al. (1996) *Nucleic Acid Res* 24, 1646-52). 2µg pGL3/utroB/F or R, or its
20 molar equivalent, mixed with 0.5µg of LacZ control plasmid (pSV-β-gal, Promega) was transfected in each well of 6 well plates using Superfect (Qiagen), following the manufacturer's protocol. 48 hours later, cells were harvested and cell extracts were assayed for luciferase and β-galactosidase
25 activity as described (Dennis, et al. (1996) *Nucleic Acids Res* 24, 1646-52). Luciferase activity was standardised to β-galactosidase activity in each individual sample to control for transfection efficiency. Results are expressed as mean luciferase/β-galactosidase ratio for four individual
30 transfections. Error bars indicate the standard error of the mean. For comparison of different constructs within the same cell line, results were standardised to those obtained with pGL3/utroB/F and are expressed as % of this value. For comparison of constructs between cell lines, results were
35 standardised to those obtained with a luciferase-SV40

promoter/enhancer plasmid (pGL3 control, Promega) that generates high levels of reporter activity in all cell lines tested.

Primer Extension

5 Primer extension was carried out as described (18); end-labelled primer BR2 was annealed to 0, 30 or 50 μ g mouse heart total RNA at 58°C for 20 minutes, and extended at 42°C for 40 minutes. Products were separated on a 6% polyacrylamide gel, under denaturing conditions, alongside a sequencing ladder
10 generated from pBSX8.0 using primer BR2.

Results

An alternative 5' exon in utrophin mRNA

Utrophin from a mouse heart cDNA library was amplified by 5'RACE, and the resulting products cloned and sequenced. Of 12
15 clones, 8 contained novel sequence 5' of exon 3. Below, we present evidence that the novel sequence is a single alternative 5' exon of utrophin containing a translational initiation codon. We refer to this sequence as 'exon 1B' to distinguish it from the previously described 5' cDNA sequence
20 comprising untranslated exon 1A and exon 2A which contains the translational start (Figure 5c).

Figure 3 shows a sequence comparison of human and mouse exon 1B, and genomic flanking sequence. The position and phase of the splice junction at the 5' end of exon 3 is identical for
25 both exon 1B- and exon 2A- containing transcripts. Exon 1B contains a putative ATG translation initiation codon and open reading frame, in-frame with that of exon 3, predicting a novel 31 amino acid N-terminus to the utrophin protein. The context of the ATG codon is predicted to be favourable for
30 translation in that there is a purine at position -3 (bold in Figure. 3) (33). Human and mouse exons 1B show 82% nucleotide identity. The predicted translations are 84% identical and 94%

similar. The position and context of the ATG codon are conserved. The human sequence contains a second putative ATG codon immediately 5' (position 1511, solid bar in Figure.1), followed by a TAG stop codon. As this ATG does not adhere to the Kozak consensus, is not associated with an open reading frame and is not present in the mouse sequence, we predict that this is not a functional translation start. A similar feature is present in human exon 2A, where the 5'UTR contains a short open reading frame prior to the true translation start.

The transcript associated with exon 1B

A human multiple tissue northern blot was probed with an exon 1B anti-sense riboprobe. A single hybridising 13kb band was observed, identical to that produced by probing the same blot with a cDNA encompassing 4kb of the utrophin C-terminus, indicating that exon1B is exclusively associated with a full-length utrophin mRNA. Exon 1B is ubiquitously expressed, and appears most abundant in heart and pancreas, and least abundant in the brain, relative to β -actin. This is similar to the expression profile of total full-length utrophin.

RT-PCR was employed to confirm the association of exon 1B with a utrophin mRNA predicted to give rise to functional protein (Figure.4). Amplification of first strand cDNA from IN157 cells utilising a forward primer specific to exon 1B (BF3I) and a reverse primer within the utrophin C-terminus (CT2) produced a product of expected size. Successive hybridisation of this PCR product with domain-specific probes; U41, UBR4, U107 and U16, confirmed that exon 1B is associated with a utrophin transcript spanning the full coding sequence of the gene.

The expression profiles of exons 1B and 2A were examined using RNase protection. Specific riboprobes corresponding to the exon 1B/3 and 2A/3 boundaries were simultaneously hybridised with total RNA, allowing direct quantitation of transcript

abundance. B-utrophin is the most abundant form in the heart, whereas exon 2A-containing transcripts predominate in the kidney. Approximately equal amounts of exons 1B and 2A were observed in the brain and in skeletal muscle.

5 **Mapping and cloning of genomic sequence associated with exon 1B**

Using probe BR4, exon 1B was mapped within our previously described human YAC contig (26) encompassing the 5' end of the utrophin locus (Figure.5a). A hybridising band was seen with
10 YAC 4X124H10 but not 4X23E3 or 5C2 indicating that exon 1B lies within the 120kb intron 2 of the utrophin gene. A subsequent database search identified a clone from the HGMP human chromosome 6 sequencing project, containing exons 1A, 2A and 1B. This indicated that exon 1B lies 52.2kb 3' of exon 2A
15 (Figure.5a). Probing the mouse genomic PAC library (RPCI21 from P. DeJong, Roswell Park Cancer Institute) with utrophin exons 1A, 1B and 2- 5 inclusive identified a series of genomic PACs spanning the 5' end of the mouse utrophin gene. Four of these PACs were assembled into a contig of the region.
20 Hybridisation with UB400 confirmed that exon 1B lies within intron 2 in the mouse (Figure.5b), approximately 50kb 3' of exon 2.

Human and mouse genomic fragments were obtained from the YAC and PAC libraries, respectively. Genomic sequence
25 encompassing exon 1B was obtained by an Xba I digest of YAC 4X124H10 (human 3kb fragment) and PAC110c24 (mouse 8.8kb fragment). These fragments were sub-cloned into pBluescript vector, the human fragment was deleted to 2kb during the sub-cloning. The plasmid clones were designated pBSX2.0 (human)
30 and pBSX8.0 (mouse). Comparison of the cDNA and genomic sequence showed no evidence of a further 5' exon in the transcript associated with exon 1B, suggesting that the genomic flanking sequence contained the transcription start and promoter element responsible for exon 1B expression. Our

nomenclature for utrophin 5' exons, transcripts and promoters appears in Figure 5c.

Promoter B

1.5kb of human genomic sequence 5' of exon 1B, including the 5'UTR of exon 1B, was cloned in both orientations into a promoterless luciferase reporter vector. Three human cell lines (IN157 rhabdomyosarcoma, CL11T47 kidney epithelial and HeLa cervical epithelial) were transiently transfected with these constructs. These three lines were chosen because they are known to express utrophin mRNA and protein at different levels. Reporter activity was detected at significantly higher levels in cells transfected with the forward than the reverse orientation construct, indicating promoter activity (Figure 6). Interestingly, the level of activity varied between cell lines by an order of magnitude. Semi-quantitative RT-PCR demonstrated that the variation of luciferase expression mimicked the transcription profile of endogenous utrophin exon 1B. In contrast, the GA3PDH control showed identical amplification in all cDNA samples, indicating that the differences seen in B-utrophin amplification have arisen from differences in the level of expression of the endogenous B-utrophin transcript in these cell lines. These data show that the 1.5kb of genomic sequence 5' of exon 1B utilised in these reporter clones contains the necessary signals to initiate transcription of exon 1B, and regulatory elements that determine the level of expression in these cell lines.

To further delineate important elements within this region, a series of 5' and 3' deletions of promoter B were made, and the *in vitro* activity of each one assayed (Figure 7). A 300bp element, contained within clone pGL3/utroB/F/D5' Pvu 1199, retains 70% activity of the full 1.5kb construct in expressing cell lines, and shows 74% identity between human and mouse (Figure.3). Homology falls to 50% when sequence further 5' if the human PvuII site is compared with corresponding mouse

sequence using a 35bp window. Homology was determined using GAP, from version 20 of GCG, with default parameters as noted already above.

Promoter B transcription start site

5 The 5' ends of 8 human and 4 mouse 5'RACE clones clustered around a putative cap site in the genomic sequence (Figure.3). None of the 5'RACE clones generated by amplification across the exon 3/exon 1B boundary extended further upstream. RT-PCR was carried out using forward primers around this region with
10 a reverse primer in exon 4. A product of expected size was amplified from IN157 cDNA by primers BF42 and BF8, but not BF16 or BF15, indicating that the transcription start is within the 18bp that separates the two primers BF15 and BF42. These 18 bases contain the putative cap site and the cluster
15 of RACE clone 5' ends.

To map the start site accurately, primer extension using an exon 1B reverse primer and mouse heart RNA was employed. This yielded a single product, indicative of a single transcription start site. Transcription initiates at mouse position 1183
20 within a 25-bp motif, which is 100% conserved between human and mouse. Part of this motif, spanning the cap site, is a 6/7 base match for the initiator consensus, and correspondingly shows homology to the initiators of other genes. The transcription start site is homologous to the initiators of
25 other promoters. Consensus 1, initiator consensus derived from sequence comparison of Inr⁺ genes (Azizkhan, et al. (1993) *Critical Reviews in Eukaryotic Gene Expression* 3, 229-254.); consensus 2, experimentally-derived consensus for functional initiator (Javahery, et al. (1994) *Molecular and Cellular*
30 *Biology* 14, 116-127.); TdT, terminal deoxynucleotidyl transferase; hRAR, human retinoic acid receptor α ; mCREB, mouse cAMP response element binding protein. Transcribed sequence is indicated in bold uppercase. We consider this promoter to be of the TATA⁻Inr⁺ type.

Assaying for substances which modulate utrophin promoter activity

Method 1:

This method uses a mouse *mdx*-H2K myoblast line stably
5 transfected with a human 7.0kb utrophin promoter-luciferase
construct. On day 1 myoblast cells transfected with the
construct are plated out in 6-well dishes, with compound or
DMSO-only for the negative controls.

4 x 6 well plates are used for every 3 compounds (the
10 compounds are dissolved in DMSO and stored prior to use). For
example, compound A, or B, or C were each added to 1 well,
while the remaining 3 wells contain only DMSO. This results
in 4 wells containing each compound and 12 wells with DMSO
alone. Due to the inherent noise of both the harvesting/assay
15 and cell seeding/growth steps, this is the minimum number that
results in meaningful analysis. Setting up the plates in this
way means that the data really are paired, and can be analysed
with a paired student T test. This provides a more powerful
statistical analysis rather than putting each compound on a
20 different plate and comparing it with a control plate.

On Day 4 the cells are harvested and luciferase quantitation
and pairwise analysis is carried out.

Method 2:

Compounds which up-regulate the endogenous utrophin promoter
25 are be found using *mdx*-H2K myoblasts that are not transfected
with the utrophin promoter-luciferase construct. *Mdx*-
myoblasts can be used to mimic utrophin transcritption and
protein stability in dystrophin-deficient cells.

Identification of utrophin protein expression

30 Quantitative Western Blotting is used to measure the level of

utrophin expression (Tinsley JM, et al., *Nature Medicine* 4, 1441-1444.) Using 6 well plates and treating with compound as described above generates enough total protein sample to test by Western blotting. Antibodies specific to the A protein or
5 B protein are used to quantify levels of either protein.

Identification of utrophin RNA expression

Quantitative ribonuclease protection is used to analyse levels of utrophin expression. A pairwise design is used, as described above, but more cells are necessary. To see bands
10 clearly, about 20-30 μ g total RNA is used. Each compound and control will need a 175 cm² tissue culture flask. A dual probe to simultaneously identify the A transcript and B transcript is be used.

Using the two techniques described compounds are identified
15 after cell treatment which modulate utrophin levels. The same techniques are used for *in vivo* animal experiments where the compound is administered to dystrophin deficient *mdx* mice.

Interleukin-6 (IL-6) Interactions

Two related elements are present in the promoters of genes
20 encoding acute phase proteins that mediate an increase in transcription stimulated by an IL-6 triggered signalling cascade (Hocke et al., 1992). One of these was found to be present in the exon 1B flanking sequence. Wild type and mutated reporter fusions for IL-6 were therefore tested for
25 responsiveness in appropriate cell systems.

Constructs of the 1.5F B promoter normal and mutant (consensus change : ctggaa > gatatc^a concerning the mutant: Hattori M et al (1990) *Proc. Natl. Acad. Sci. U S A.* Mar;87(6):2364-8.) were introduced into a promoter-less luciferase reporter vector and
30 transfected into IN157 cells with a renilla firefly control. Cells were washed and charcoal stripped serum added 5 hours

post-transfection and left overnight. IL-6 amounts were added as illustrated with an appropriate amount of IL-6 soluble receptor. The cells were left for 24 hours and then assayed for activity using a luminometer.

- 5 A dosage dependent transcriptional response was noted in the normal, but not the mutated reporter construct (figure 10). This result indicates the existence of a cytokine mediated signalling pathway which causes up-regulation of the B utrophin promoter through the interaction of IL-6 and IL-6 receptor with
10 the conserved IL-6 response element.

Discussion

We have demonstrated that there is a second promoter within intron 2 of the utrophin gene, driving expression of a unique first exon that splices into a common 13kb mRNA. These data are
15 important, both in terms of understanding the molecular physiology of utrophin expression, and in view of their application to therapeutic intervention in DMD.

The functional consequences of genes having more than one promoter have been postulated (reviewed in (Ayoubi, et al
20 (1996) *FASEB J.* 10,453-460). A single gene may achieve a complex temporal and spatial expression pattern by interaction of different promoters with discrete subsets of transcription factors. Dystrophin is an example: three dissimilar promoters are active at different levels in specific cell types within
25 the heart, skeletal muscle and the brain (Gorecki, et al. (1992) *Hum Mol Genet* 1, 505-510., Barnea, et al. (1990) *Neuron* 5, 881-888, Holder, et al. *Human Genetics* 97, 232-239). Northern blot analysis, however, indicates that utrophin exon 1B is ubiquitously expressed, implying that promoters A and B
30 are co-expressed in many tissues. It is conceivable that examination of transcript distribution in whole tissue samples has masked cell type-specific patterns of expression. Data

from isolated human cell lines *in vitro* support this notion; we observed large differences in promoter B activity between different cell lines, consistent with an *in vivo* expression profile involving specific cellular populations.

5 Alternatively, the two promoters may be spatially regulated at a sub-cellular level. Within adult skeletal muscle fibres, promoter A is synaptically driven (Gramolini, et al. (1997) *J Biol Chem* 272, 8117-20.), yet aggregates of utrophin mRNA are detectable at up to 25% extrasynaptic nuclei (Vater, et al.
10 (1998) *Molecular and cellular Neuroscience* 10, 229-242). Expression of promoter B in the extrasynaptic compartment might be invoked as one possible explanation.

A second proposed function of alternative promoters is the generation of transcripts with interchangeable 5' exons,
15 giving rise to mRNAs with alternative 5'UTRs or proteins with novel N-terminal domains. Unlike exon 1B, utrophin exon 1A contains a long GC-rich 5'UTR. In some transcripts, GC-rich 5'UTRs are not translated efficiently (Kozak, M. (1991) *J Cell Biol* 115, 887-903.), and there are examples of genes in which
20 alternative use of GC-rich and non-GC-rich 5'UTRs has been implicated in post-transcriptional regulation of protein synthesis (Nielson, et al. (1990) *J Biol Chem* 265, 13431-13434.). In addition, the predicted 31 amino acids encoded by exon 1B are different to the 26 amino acids of exon 2A; the
25 functions of the resulting N-termini may be different.

The discovery of a second promoter provides a new target for the upregulation of utrophin to ameliorate the DMD phenotype. Promoter B is highly regulated, probably by different factors from promoter A, including IL-6. Elucidation of the mechanisms
30 responsible for the large difference in promoter B activity between IN157 and HeLa cells might lead to identification of a factor that can be delivered to muscle to activate utrophin expression. Importantly, as the N-box motif is absent from promoter B, this is unlikely to carry any risk of NMJ

disruption potentially inherent in the pharmacological manipulation of synaptically regulated promoter A.

CLAIMS

1. An isolated nucleic acid comprising a promoter which comprises a sequence of nucleotides selected from (i) the human promoter sequence shown in Figure 1 and (ii) the mouse promoter sequence shown in Figure 2, free or substantially free of utrophin coding sequence.
2. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1440 in Figure 1.
3. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1183 of the mouse sequence shown in Figure 2.
4. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 1199 -1440 in the sequence shown in Figure 1.
5. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 959-1183 in the sequence shown in Figure 2.
6. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotide sequence
ACAGGACATCCCAGTGTGCAGTTCG.
7. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 1, which sequence has at least 60% homology with the promoter sequence shown in figure 1 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that

sequence, said transcription being muscle-specific.

8. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion,
5 deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which sequence has at least 60% homology with the promoter sequence shown in figure 2 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that
10 sequence, said transcription being muscle-specific.

9. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the
15 promoter sequence shown in Figure 2, which hybridises to the promoter sequence shown in figure 2 under stringent hybridisation conditions and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription
20 being muscle-specific.

10. A nucleic acid construct comprising an isolated nucleic acid according to any of the preceding claims operably linked to a heterologous sequence.

11. A nucleic acid construct according to claim 10 wherein
25 the heterologous sequence is a coding sequence.

12. A nucleic acid construct according to claim 11 wherein the heterologous sequence encodes a reporter molecule.

13. A host cell comprising a nucleic acid construct according to any of claims 10 to 12.

14. A method comprising culturing a host cell according to claim 13 under conditions for transcription of said heterologous sequence from the promoter.

5 15. A method according to claim 14 wherein the heterologous sequence is a coding sequence and the host cell is cultured under conditions for expression of the encoded peptide or polypeptide product.

16. A method according to claim 14 or claim 15 comprising detection of transcription of the heterologous sequence.

10 17. A method according to claim 14 or claim 15 comprising detection of expression of the encoded peptide or polypeptide product.

15 18. A method of screening for a substance able to modulate utrophin promoter activity, the method comprising contacting an expression system containing a nucleic acid construct according to any of claims 10 to 12 with a test or candidate substance and determining transcription of the heterologous sequence or expression of the encoded peptide or polypeptide product.

20 19. A method according to claim 18 wherein the expression system comprises a host cell containing said nucleic acid construct.

25 20. A method which comprises, following identification of a substance able to modulate utrophin promoter activity in accordance with a method according to claim 18 or claim 19, manufacture of the substance and/or use of the substance in manufacture or formulation of a composition.

30 21. The use of an isolated nucleic acid according to any of claims 1 to 6 for promoting transcription of an operably

linked sequence of nucleotides.

22. The use of claim 21 wherein the transcription is tissue-specific, with the tissue-specificity being muscle-specific.

5 23. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide including the amino acid sequence shown in Figure 1 or Figure 2.

10 24. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide including the amino acid sequence shown in Figure 1, which amino acid sequence has at least 60% homology with the polypeptide sequence in Figure 1 or Figure 2.

15 25. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide shown in Figure 1 or Figure 2, which nucleotide sequence hybridises with the nucleotide sequence encoding the polypeptide in Figure 1 or Figure 2 under stringent hybridisation conditions.

20 26. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence shown in Figure 9.

25 27. An isolated nucleic acid molecule comprising the nucleotide sequence shown in figure 9.

28. Nucleic acid of any one of claims 23 to 27 comprised in a vector.

29. Nucleic acid according to claim 28 wherein said vector is an expression vector.

30. A host cell containing heterologous nucleic acid according to any one of claims 23 to 29.

31. A cell according to claim 30 which is a muscle cell.

32. A cell according to claim 30 wherein said polypeptide
5 is expressed.

33. A cell according to any of claims 30 to 32 which is in a mammal.

34. A non-human mammal having a cell according to any of claims 30 to 32.

10 35. A non-human mammal containing nucleic acid according to any of claims 23 to 29.

36. A method including introduction of nucleic acid according to any of claims 23 to 29 into a cell.

15 37. A method according to claim 36 wherein said introduction takes place *in vitro*.

38. A method which includes causing or allowing expression of the coding nucleotide sequence of heterologous nucleic acid according to any of claims 23 to 29 in a cell.

20 39. A method according to claim 38 wherein the cell is part of a mammal.

40. A method according to claim 38 wherein the expression product is purified and/or isolated following expression.

41. A method according to claim 40 wherein the expression product is formulated into a composition which includes at
25 least one additional component, following purification and/or

isolation of the expression product.

42. An isolated polypeptide as encoded by nucleic acid according to any of claims 23 to 29.

43. An isolated utrophin exon IB polypeptide selected from:

5 (i) human utrophin exon IB polypeptide of which the amino acid sequence is shown in Figure 1;

(ii) mouse utrophin exon IB of which the amino acid sequence is shown in Figure 1.

10 44. An isolated polypeptide including the human polypeptide according to claim 43.

45. An isolated polypeptide including the mouse polypeptide according to claim 44.

15 46. An isolated polypeptide which has 60 % homology with the polypeptide according to claim 44 or 45.

47. An isolated fragment of a polypeptide according to claim 43, which fragments is 5 to 25 amino acids in length.

48. An isolated fragment of a polypeptide according to claim 43, which fragment is 10 to 20 amino acids in length.

20 49. An antibody specific for a polypeptide according to any one of claims 42 to 48.

50. A composition including a polypeptide according to any one of claims 42 to 46, a fragment according to claim 47 or claim 48, or an antibody according to claim 49, and a
25 pharmaceutically acceptable excipient.

51. Use of nucleic acid according to any of claims 23 to 29 in the manufacture of a medicament for treating a dystrophin

phenotype in a mammal.

52. Use of a polypeptide according to any of claims 42 to 48 or an antibody according to claim 49 in the manufacture of a medicament for treating a dystrophin phenotype in a mammal.

[illegible]

Figure 1

1199 tactacgtgggttatagcagtaaacagggttttgactaagtgcacatgactggagccattctgattctttactgtctcaccacattatttcggttgaggatgaggatcagaggacagantgcttagtggtttttccagagtcctcaagt 338

339 ctatggtctctgagctacatagataggttcccttttaettggaactcctgtggacccgtgtaggttaacatatctgtgagaatccttgtgctaggtacggattctgtttcagaggaggaaagaaagctatttagatccatactactaaga 486

487 tgcaggcatggcagatcaaacacaccttccctctctctttttgcacgtgtgtgtagagaaacacatctgcaaatgatgtgtagagagacacaaacacacacatctaaaacagagaagtctggaaataatgagctctgtgtgggtattatgttttttcca 630

631 cccatgacacattcttctcctttctcttctcttatgaatatttttgagadacaagaatttctctatatagacctagctgtctgtggactttacactgtagaccaggctggccttgaaactcacagatgccacctgccttccttc

repetitive elements

765 ctatctgagdgataaaacgcgtgtaccacacacaagaatcaaacactgttttgadtatgatagtgggggtgtgttgtgtgtgtgtcgaacaccatacaacagaaaaattageatctctctagtgtaccttgg 898

CA repeat

silencer/CdxA
 899 aacattcgaagctcraagctgtgacratataaaaccaaagtcctcaagagttcttaactgactgcggaggttaactctctgctgaggagagtcggagtgattagtccagatcctctctggtggaaaaatacaagg 1039
 C-ets1/PEN3/XrpF1 Spl AP1
 CACbp

TATA cap M S L A A T T F
Initiator
1040 gactttaaaagaataaaccttaacaggatccccagtcgagttccggcggcggttttcttgatgttccctcatctcagccactgttaggtgagcaggcctggcagccaccacatttt 1183

R W K K W R L D L P G Q V P L Q A C R R S P D splice to exon 3
1184 CGTTGGAAAGTGGAGGTGAGCTGCCTTGGGAGAGGCCCTTCCTCAGGCTTCACAGGAGATCCCCCGCAAGTTGTCAGTGGCCAGACTGCAGTTGCTAAGGAGGAGCTTGGACAGAGGgTgtcaggttagccacagagggtgttcaggttagccacacttcttc splice donor

Figure 2

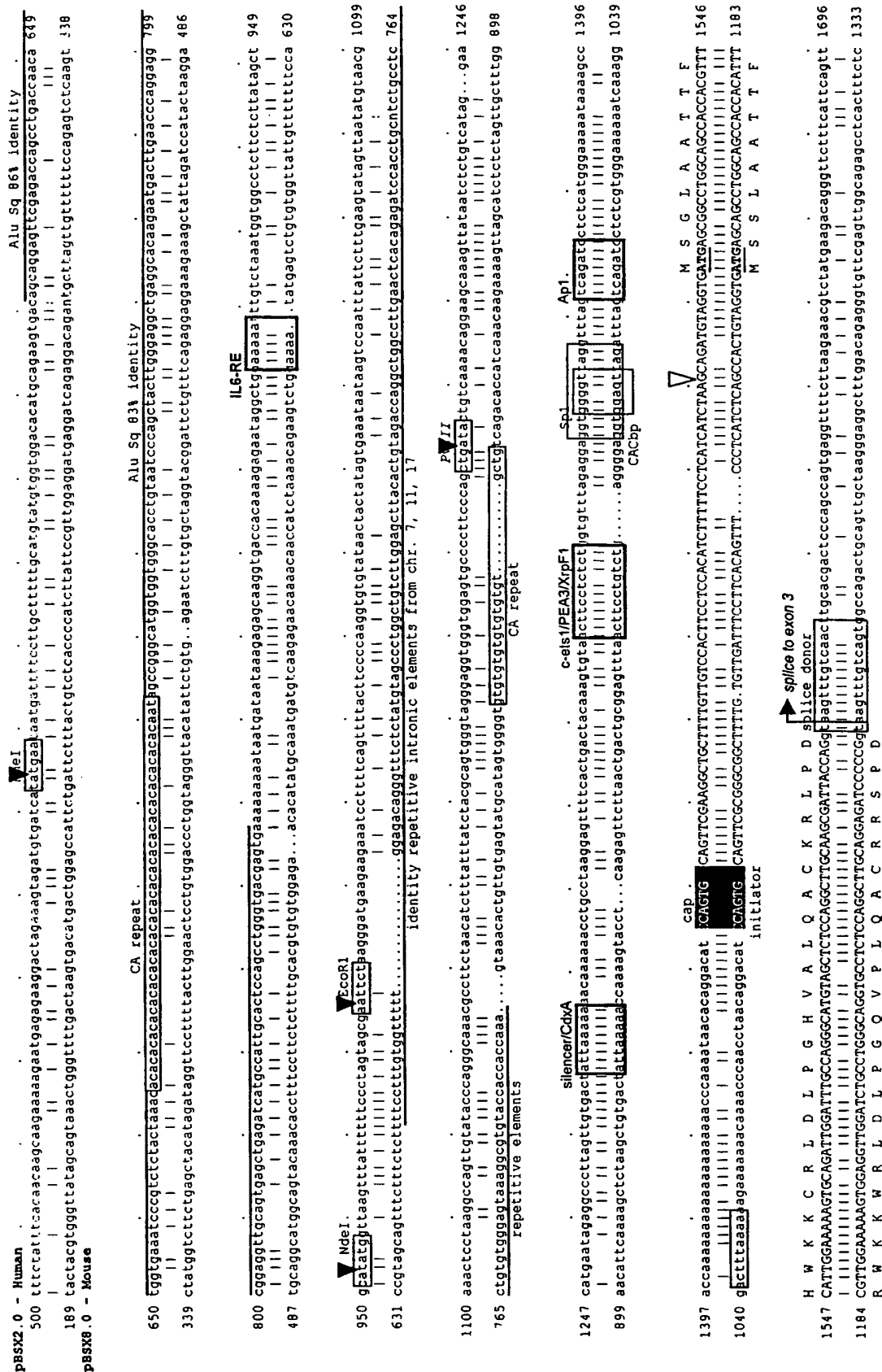


Figure 3

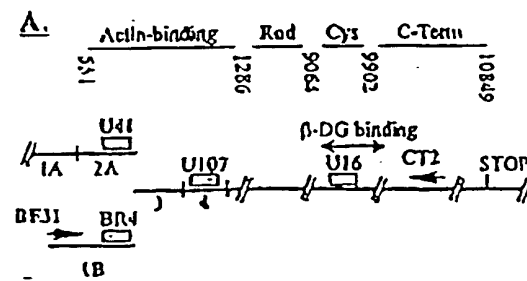


Figure 4

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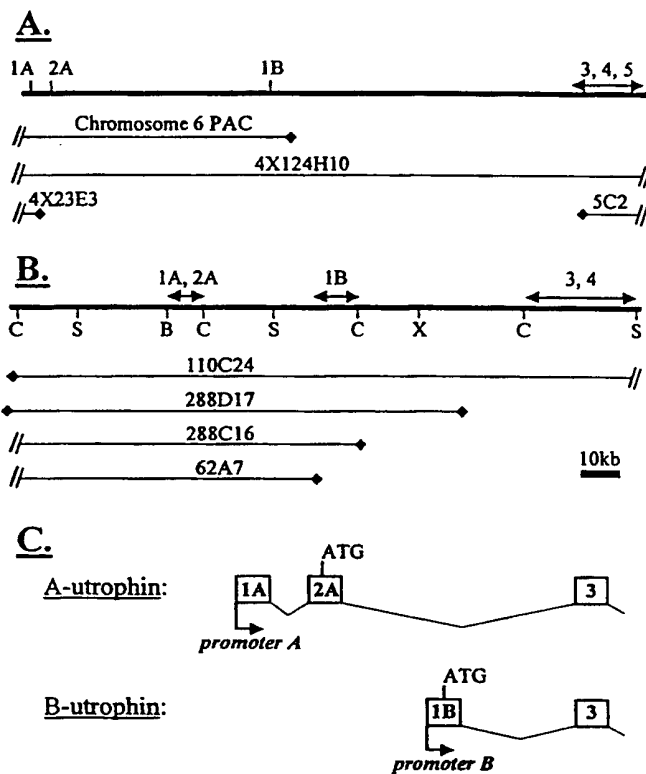


Figure 5

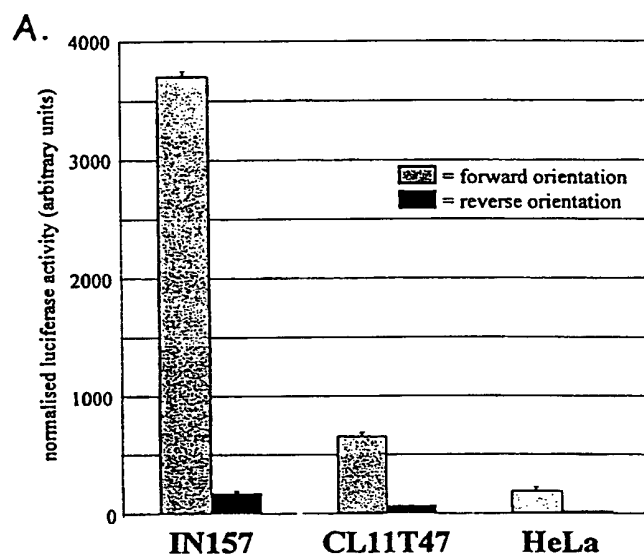


Figure 6

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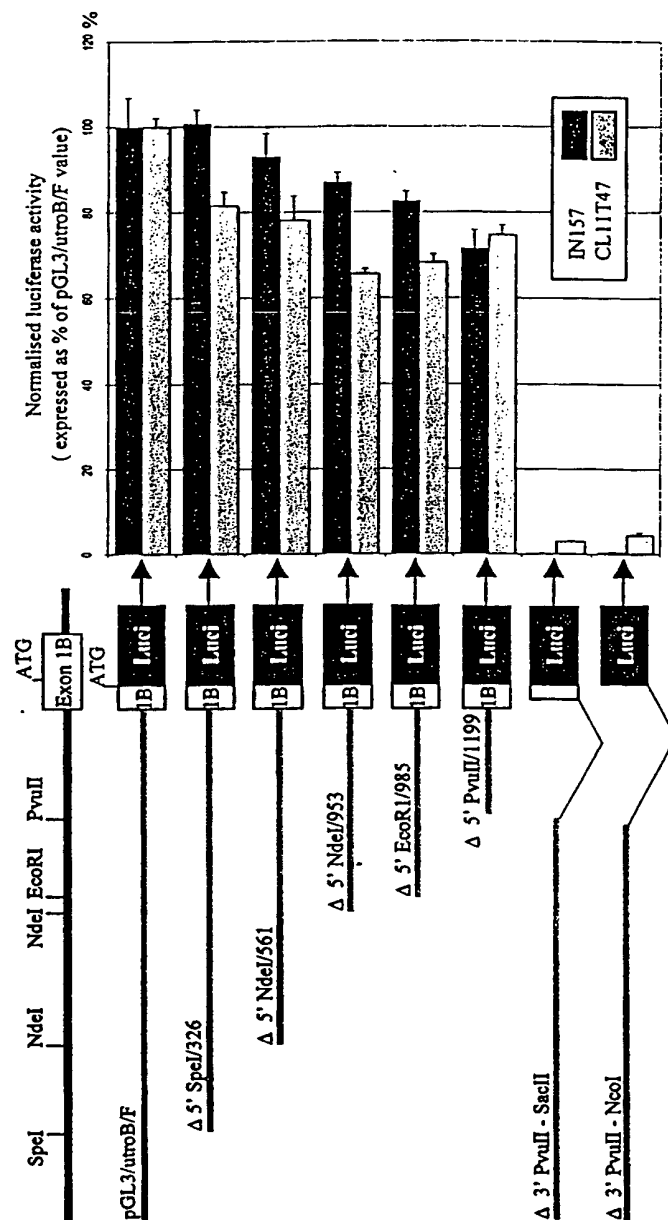


Figure 7

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Human B-utrophin up to nucleotide 1500, deduced translation

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CCCAGTGTGCAGTTCGAAGGCTGCTTTTGTGTCCACTTCCTCCACATCTTTTCCTCAT
1 -----+-----+-----+-----+-----+-----+-----+-----+ 60
GGGTACACAGTCAAGCTTCCGACGAAAACAACAGGTGAAGGAGGTGTAGAAAAAGGAGTA

CATCTAAGCAGATGTAGGTGATGAGCGGCCTGGCAGCCACCACGTTTCATTGGAAAAAGT
61 -----+-----+-----+-----+-----+-----+-----+-----+ 120
GTAGATTGCTCTACATCCACTACTCGCCGGACCGTCGGTGGTGCAAAGTAACCTTTTCA

M S G L A A T T F H W K K C -

Exon 1B ←-----→ Exon 3

GCAGATTGGATTGGCCAGGGCATGTAGCTCTCCAGGCTTGCAAGCGATTACCAGATGAAC
121 -----+-----+-----+-----+-----+-----+-----+-----+ 180
CGTCTAACCTAAACGGTCCCGTACATCGAGAGGTCCGAACGTTGCTAATGGTCTACTTG

c      R L D L P G H V A L Q A C K R L P D E H -

ACAATGACGTACAGAAGAAAACCTTTACCAAATGGATAAATGCTCGATTTTCAAAGAGTG
181 -----+-----+-----+-----+-----+-----+-----+-----+ 240
TGTTACTGCATGTCTTTTGGAAATGGTTTACCTATTACGAGCTAAAAGTTTCTCAC

c      N D V Q K K T F T K W I N A R F S K S G -

GGAAACCACCCATCAATGATATGTTACAGACCTCAAAGATGGAAGGAAGCTATTGGATC
241 -----+-----+-----+-----+-----+-----+-----+-----+ 300
CCTTTGGTGGGTAGTTACTATACAAGTGTCTGGAGTTTCTACCTTCCTTCGATAACCTAG

c      K P P I N D M F T D L K D G R K L L D L -

TTCTAGAAGGCCTCACAGGAACATCACTGCCAAGGAACGTGGTTCCACAAGGTTACATG
301 -----+-----+-----+-----+-----+-----+-----+-----+ 360
AAGATCTTCCGGAGTGTCTTGTAGTGACGGTTTCTTGCACCAAGGTGTTCCCATGTAC

c      L E G L T G T S L P K E R G S T R V H A -

CCTTAAATAACGTCAACAGAGTGCTGCAGGTTTTACATCAGAACAATGTGGAATTAGTGA
361 -----+-----+-----+-----+-----+-----+-----+-----+ 420
GGAATTTATTGCAGTTGTCTCACGACGTCCAAAATGTAGTCTTGTACACCTTAATCACT

c      L N N V N R V L Q V L H Q N N V E L V N -

ATATAGGGGGAAGTACATTGTGGATGGAAATCACAAGTACTTTGGGGTTACTTTGGA
421 -----+-----+-----+-----+-----+-----+-----+-----+ 480
TATATCCCCCTTGACTGTAACACCTACCTTTAGTGTGTTGACTGAAACCCCAATGAAACCT

c      I G G T D I V D G N H K L T L G L L W S -

GCATCATTTTGCAGTGGCAGGTGAAAGATGTATGAAGGATGTATGTCGGACCTGCAGC
481 -----+-----+-----+-----+-----+-----+-----+-----+ 540
CGTAGTAAAACGTGACCGTCCACTTTCTACAGTACTTCTACAGTACAGCCTGGACGTCG

c      I I L H W Q V K D V M K D V M S D L Q Q -

AGACGAACAGTGAGAAGATCCTGCTCAGCTGGGTGCGTCAGACCACCAGGCCCTACAGCC
541 -----+-----+-----+-----+-----+-----+-----+-----+ 600
TCTGCTTGTCAGTCTTCTAGGACGAGTCGACCCAGCAGTCTGGTGGTCCGGGATGTCGG

c      T N S E K I L L S W V R Q T T R P Y S Q -

AAGTCAACGTCTCAACTTCACCACCAGCTGGACAGATGGACTCGCCTTTAATGCTGTCC
601 -----+-----+-----+-----+-----+-----+-----+-----+ 660
TTCAGTTGCAGGAGTTGAAGTGGTGGTCGACCTGTCTACCTGAGCGGAAATTACGACAGG

c      V N V L N F T T S W T D G L A F N A V L -

TCCACCGACATAAACCTGATCTCTTCAGCTGGGATAAAGTTGTCAAATGTCACCAATTG
661 -----+-----+-----+-----+-----+-----+-----+-----+ 720
AGGTGGCTGTATTTGGACTAGAGAAGTCGACCCTATTTCAACAGTTTACAGTGGTTAAC

c      H R H K P D L F S W D K V V K M S P I E -

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Figure 8



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AGAGACTTGAACATGCCTTCAGCAAGGCTCAAACCTATTGGGAATTGAAAAGCTGTTAG
721 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 780
TCTCTGAACCTTGTACGGAAGTCGTTCCGAGTTTGAATAAACCTTAACCTTTTCGACAATC

c      R L E H A F S K A Q T Y L G I E K L L D -

ATCCTGAAGATGTTGCCGTTCCGCTTCCTGACAAGAAATCCATAATTATGTATTTAACAT
781 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 840
TAGGACTTCTACACGGCAAGCCGAAGGACTGTTCTTTAGGTATTAATACATAAAATTGTA

c      P E D V A V R L P D K K S I I M Y L T S -

CTTTGTTTGAGGTGCTACCTCAGCAAGTCACCATAGACGCCATCCGTGAGGTAGAGACAC
841 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 900
GAAACAACTCCACGATGGAGTCGTTTCAGTGGTATCTGCGGTAGGCACTCCATCTCTGTG

c      L F E V L P Q Q V T I D A I R E V E T L -

TCCCAAGGAAATATAAAAAAGAATGTGAAGAAGAGGCAATTAATATACAGAGTACAGCGC
901 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 960
AGGGTTCCTTTATATTTTCTTACACTTCTTCTCCGTTAATTATATGTCTCATGTGCGG

c      P R K Y K K E C E E E A I N I Q S T A P -

CTGAGGAGGAGCATGAGAGTCCCGAGCTGAAACTCCCAGCACTGTCACTGAGGTGCGACA
961 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1020
GACTCCTCCTCGTACTCTCAGGGGCTCGACTTTGAGGGTCGTGACAGTGACTCCAGCTGT

c      E E E H E S P R A E T P S T V T E V D M -

TGGATCTGGACAGCTATCAGATTGCGTTGGAGGAAGTGTGACCTGGTTGCTTTTGCTG
1021 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1080
ACCTAGACCTGTCGATAGTCTAACGCAACCTCCTTCACGACTGGACCAACGAAAGACGAC

c      D L D S Y Q I A L E E V L T W L L S A E -

AGGACACTTTCCAGGAGCAGGATGATATTTCTGATGATGTTGAAGAAGTCAAAGACCAGT
1081 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1140
TCCTGTGAAAGTCCCTCGTCTACTATAAAGACTACTACAATTCTTCAGTTTCTGGTCA

c      D T F Q E Q D D I S D D V E E V K D Q F -

TTGCAACCCATGAAGCTTTTATGATGGAAGTACTGACACACCAGAGCAGTGTGGGCAGCG
1141 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200
AACGTTGGGTACTTCGAAATACTACCTTGACTGACGTGTGGTCTCGTACACCCGTCGC

c      A T H E A F M M E L T A H Q S S V G S V -

TCCTGCAGGCAGGCAACCAACTGATAACACAAGGAAGTCTGTCAGACGAAGAAGATTG
1201 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1260
AGGACGTCGTCGTTGGTTGACTATTGTGTTCTTGGAGACAGTCTGCTTCTTCTTAAC

c      L Q A G N Q L I T Q G T L S D E E E F E -

AGATTCAAGAACAGATGACCTGCTGAATGCTAGATGGGAGGCTCTTAGGGTGGAGAGTA
1261 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1320
TCTAAGTCCTTGCTACTGGGACGACTTACGATCTACCCTCCGAGAATCCCACCTCTCAT

c      I Q E Q M T L L N A R W E A L R V E S M -

TGGACAGACAGTCCCGCTGCACGATGTGCTGATGGAAGTGCAGAAGAAGCAACTGCAGC
1321 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1380
ACCTGTCTGTGAGGGCCGACGTGCTACACGACTACCTTGACGTCTTCTTCGTTGACGTGC

c      D R Q S R L H D V L M E L Q K K Q L Q Q -

AGCTCTCCGCCTGGTTAACTCAGAGAGGAGCGCATTGAGAAGATGGAACTTGCCCCC
1381 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1440
TCGAGAGGCGGACCAATTGTGAGTGTCTCCTCGCGTAAGTCTTCTACCTTTGAACGGGG

c      L S A W L T L T E E R I Q K M E T C P L -

TGGATGATGATGTAATCTCTACAAAAGCTGCTAGAAGAACATAAAAGTTTGCAAAGTG
1441 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1500
ACCTACTACTACATTTAGAGATGTTTCGACGATCTTCTGTATTTTCAAACGTTTCAC

c      D D D V K S L Q K L L E E H K S L Q S D -

```

Figure 8 cont ...

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Sequence Range: 1 to 6059

```

      10      20      30      40      50      60      70      80
ACTAGTCAAG ATGAGCGGCC TGGCAGCCAC CACGTTTCAT TGGAAAAAGT GCAGATTGGA TTTGCCAGGG CATGTAGCTC
      M S G L A A T T F H W K K C R L D L P G H V A>

      90     100     110     120     130     140     150     160
TCCAGGCTTG CAAGCGATTA CCAGATGAAC ACAATGATGT ACAGAAGAAA ACCTTTACCA AATGGATAAA CGCTCGATT
L Q A C K R L P D E H N D V Q K K T F T K W I N A R F>

      170     180     190     200     210     220     230     240
TCCAAGAGTG GGAAACCACC CATCAGTGAT ATGTTCTCAG ACCTCAAAGA TGGGAGAAAG CTCTTGATC TTCTCGAAG
S K S G K P P I S D M F S D L K D G R K L L D L L E G>

      250     260     270     280     290     300     310     320
CCTCACAGGA ACATCATTGC CAAAGGAACG TGGTTCACAC AGGGTGCAATG CCTTAAACAA TGTCAACCGA GTGCTACAG
L T G T S L P K E R G S T R V H A L N N V N R V L Q>

      330     340     350     360     370     380     390     400
TTTACATCA GAACAATGTG GACTTGGTGA ATATTGGAGG CACGGACATT GTGGCTGGAA ATCCCAAGCT GACTTTAGG
V L H Q N N V D L V N I G G T D I V A G N P K L T L G>

      410     420     430     440     450     460     470     480
TTACTCTGGA GCATCATTCT GCACTGGCAG GTGAAGGATG TCATGAAAGA TATCATGTCA GACCTGCAGC AGACAAACAG
L L W S I I L H W Q V K D V M K D I M S D L Q Q T N S>

      490     500     510     520     530     540     550     560
CGAGAAGATC CTGCTGAGCT GGGTGCGGCA GACCACCAGG CCCTACAGTC AAGTCAACGT CCTCAACTTC ACCACCAGCT
E K I L L S W V R Q T T R P Y S Q V N V L N F T T S>

      570     580     590     600     610     620     630     640
GGACCGATGG ACTCGCGTTC AACGCCGTGC TCCACCGGCA CAAACCAGAT CTCTTCGACT GGGACGAGAT GGTCAAATG
W T D G L A F N A V L H R H K P D L F D W D E M V K M>

      650     660     670     680     690     700     710     720
TCCCAATG AGAGACTTGA CCATGCTTTT GACAAGGCC ACACCTCTTT GGGAATTGAA AAGCTCCTAA GTCCTGAAAC
S P I E R L D H A F D K A H T S L G I E K L L S P E T>

      730     740     750     760     770     780     790     800
TGTTGCTGTG CATCTCCCTG ACAAGAAATC CATAATATG TATTTAACGT CTCTGTTTGA GGTGCTTCTT CAGCAAGTCA
V A V H L P D K K S I I M Y L T S L F E V L P Q Q V>

      810     820     830     840     850     860     870     880
CGATAGATGC CATCCGAGAG GTGGAGACTC TCCCAAGGAA GTATAAGAAA GAATGTGAAG AGGAAGAAAT TCATATCCAG
T I D A I R E V E T L P R K Y K K E C E E E E I H I Q>

      890     900     910     920     930     940     950     960
AGTGCAGTGC TGGCAGAGGA AGGCCAGAGT CCCCAGAGCT AGACCCCTAG CACCGTCACT GAAGTGGACA TGGATTGGA
S A V L A E E G Q S P R A E T P S T V T E V D M D L D>

      970     980     990     1000    1010    1020    1030    1040
CAGCTACCAG ATAGCGCTAG AGGAAGTGCT GACGTGGCTG CTGTCCGCGG AGGACACGTT CCAGGAGCAA CATGACATTT
S Y Q I A L E E V L T W L L S A E D T F Q E Q H D I>

      1050    1060    1070    1080    1090    1100    1110    1120
CTGATGATGT CGAAGAAGTC AAAGAGCAGT TTGCTACCCA TGAAACTTTT ATGATGGAGC TGACAGCACA CCAGAGCAGC
S D D V E E V K E Q F A T H E T F M M E L T A H Q S S>

      1130    1140    1150    1160    1170    1180    1190    1200
GTGGGGAGCG TCCTGCAGGC TGGCAACCAG CTGATGACAC AAGGGACTCT GTCCAGAGAG GAGGAGTTTG AGATCCAGGA
V G S V L Q A G N Q L M T Q G T L S R E E E F E I Q E>

      1210    1220    1230    1240    1250    1260    1270    1280
ACAGATGACC TTGCTGAATG CAAGGTGGGA GGCCTCCGG GTGGAGAGCA TGGAGAGGCA GTCCCGGCTG CACGACGCTC
Q M T L L N A R W E A L R V E S M E R Q S R L H D A>

      1290    1300    1310    1320    1330    1340    1350    1360
TGATGGAGCT GCAGAAGAAA CAGCTGCAGC AGCTCTCAAG CTGGCTGGCC CTCACAGAAG AGCGCATTCA GAAGATGGAG

```

Figure 9

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L M E L Q K K Q L Q Q L S S W L A L T E E R I Q K M E>
 1370 1380 1390 1400 1410 1420 1430 1440
 AGCCTCCCGC TGGGTGATGA CCTGCCCTCC CTGCAGAAGC TGCTTCAAGA ACATAAAAGT TTGCAAAATG ACCTTGAAGC
 S L P L G D D L P S L Q K L L Q E H K S L Q N D L E A>
 1450 1460 1470 1480 1490 1500 1510 1520
 TGAACAGGTG AAGGTAAATT CCTTAACTCA CATGGTGGTG ATTGTGGATG AAAACAGTGG GGAGAGTGCC ACAGCTCTTC
 E Q V K V N S L T H M V V I V D E N S G E S A T A L>
 1530 1540 1550 1560 1570 1580 1590 1600
 TGGAAGATCA GTTACAGAAA CTGGGTGAGC GCTGGACAGC TGTATGCCGC TGGACTGAAG AACGTTGGAA CAGGTGCAAA
 L E D Q L Q K L G E R W T A V C R W T E E R W N R L Q>
 1610 1620 1630 1640 1650 1660 1670 1680
 GAAATCAGTA TTCTGTGGCA GGAATTATTG GAAGAGCAGT GTCTGTTGGA GGCTTGGCTC ACCGAAAAGG AAGAGGCTTT
 E I S I L W Q E L L E E Q C L L E A W L T E K E E A L>
 1690 1700 1710 1720 1730 1740 1750 1760
 GGATAAAGTT CAAACCAGCA ACTTTAAAGA CCAGAAGGAA CTAAGTGTC A GTGTCCGGCG TCTGGCTATA TTGAAGGAAG
 D K V Q T S N F K D Q K E L S V S V R R L A I L K E>
 1770 1780 1790 1800 1810 1820 1830 1840
 ACATGGAAAT GAAGAGGCAG ACTCTGGATC AACTGAGTGA GATTGGCCAG GATGTGGGCC AATTACTCAG TAATCCCAAG
 D M E M K R Q T L D Q L S E I G Q D V G Q L L S N P K>
 1850 1860 1870 1880 1890 1900 1910 1920
 GCATCTAAGA AGATGAACAG TGA CTCTGAG GAGCTAACAC AGAGATGGGA TTCTCTGTT CAGAGACTCG AAGACTCTTC
 A S K K M N S D S E E L T Q R W D S L V Q R L E D S S>
 1930 1940 1950 1960 1970 1980 1990 2000
 TAACCAGGTG ACTCAGGCGG TAGCGAAGCT CGGCATGTCC CAGATTCCAC AGAAGGACCT ATTGGAGACC GTTCATGTGA
 N Q V T Q A V A K L G M S Q I P Q K D L L E T V H V>
 2010 2020 2030 2040 2050 2060 2070 2080
 GAGAACAAGG GATGGTGAAG AAGCCCAAGC AGGAACTGCC TCCTCCGTTA ACAAAGGCTG AGCATGCTAT GCAAAAGAGA
 R E Q G M V K K P K Q E L P P P L T K A E H A M Q K R>
 2090 2100 2110 2120 2130 2140 2150 2160
 TCAACCACCG AATGGGAGA AAACCTGCAA GAATTAAGAG ACTTAAGTCA AGAAATGGAA GTACATGCTG AAAAACTCAA
 S T T E L G E N L Q E L R D L T Q E M E V H A E K L K>
 2170 2180 2190 2200 2210 2220 2230 2240
 ATGGCTGAAT AGAACTGAAT TGGAGATGCT TTCAGATAAA AGTCTGAGTT TACCTGAAAG GGATAAAATT TCAGAAGCT
 W L N R T E L E M L S D K S L S L P E R D K I S E S>
 2250 2260 2270 2280 2290 2300 2310 2320
 TAAGGACTGT AAATATGACA TGGAAATAAGA TTGCAGAGA GGTGCCTACC ACCCTGAAGG AATGCATCCA GGAGCCCACT
 L R T V N M T W N K I C R E V P T T L K E C I Q E P S>
 2330 2340 2350 2360 2370 2380 2390 2400
 TCTGTTTCAC AGACAAGGAT TGCTGCTCAT CCTAATGTCC AAAAGGTGGT GCTAGTATCA TCTGCGTCAG ATATTCTGT
 S V S Q T R I A A H P N V Q K V V L V S S A S D I P V>
 2410 2420 2430 2440 2450 2460 2470 2480
 TCAGTCTCAT CGTACTTCGG AAATTTCAAT TCCTGCTGAT CTTGATAAAA CTATAACAGA ACTAGCCGAC TGGCTGGTAT
 Q S H R T S E I S I P A D L D K T I T E L A D W L V>
 2490 2500 2510 2520 2530 2540 2550 2560
 TAATCGACCA GATGCTGAAG TCCAACATG TCACTGTTGG GGATGTAGAA GAGATCAATA AGACCGTTTC CCGAATGAAA
 L I D Q M L K S N I V T V G D V E E I N K T V S R M K>
 2570 2580 2590 2600 2610 2620 2630 2640
 ATTACAAAGG CTGACTTAGA ACAGCGCCAT CCTCAGCTGG ATTATGTTTT TACATGGCA CAGAATTTGA AAAATAAAGC
 I T K A D L E Q R H P Q L D Y V F T L A Q N L K N K A>
 2650 2660 2670 2680 2690 2700 2710 2720
 TTCCAGTTCA GATATGAGAA CAGCAATTAC AGAAAAATTG GAAAGGGTCA AGAACCAGTG GGATGGCACC CAGCATGGCG

Figure 9 cont ...

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S S S D M R T A I T E K L E R V K N Q W D G T Q H G>

2730 2740 2750 2760 2770 2780 2790 2800
 TTGAGCTAAG ACAGCAGCAG CTGAGGACA TGATTATTGA CAGTCTTCAG TGGGATGACC ATAGGGAGGA GACTGAAGAA
 V E L R Q Q Q L E D M I I D S L Q W D D H R E E T E E>

2810 2820 2830 2840 2850 2860 2870 2880
 CTGATGAGAA AATATGAGGC TCGACTCTAT ATTCTTCAGC AAGCCCCGACG GGATCCACTC ACCAAACAAA TTTCTGATAA
 L M R K Y E A R L Y I L Q Q A R R D P L T K Q I S D N>

2890 2900 2910 2920 2930 2940 2950 2960
 CCAAATACTG CTCAAGAAC TGGGTCCTGG AGATGGTATC GTCATGGCGT TCGATAACGT CCTGCAGAAA CTCCTGGAGG
 Q I L L Q E L G P G D G I V M A F D N V L Q K L L E>

2970 2980 2990 3000 3010 3020 3030 3040
 AATATGGGAG TGATGACACA AGGAATGTGA AAGAAACCAC AGAGTACTTA AAAACATCAT GGATCAATCT CAAACAAAGT
 E Y G S D D T R N V K E T T E Y L K T S W I N L K Q S>

3050 3060 3070 3080 3090 3100 3110 3120
 ATTGCTGACA GACAGAACGC CTTGGAGGCT GAGTGGAGGA CGGTGCAGGC CTCTCGCAGA GATCTGGAAA ACTTCCTGAA
 I A D R Q N A L E A E W R T V Q A S R R D L E N F L K>

3130 3140 3150 3160 3170 3180 3190 3200
 GTGGATCCAA GAAGCAGAGA CCACAGTGAA TGTGCTTGTG GATGCCTCTC ATCGGGAGAA TGCTCTTCAG GATAGTATCT
 W I Q E A E T T V N V L V D A S H R E N A L Q D S I>

3210 3220 3230 3240 3250 3260 3270 3280
 TGGCCAGGGA ACTCAAACAG CAGATGCAGG ACATCCAGGC AGAAATTGAT GCCACAATG ACATATTAA AAGCATTGAC
 L A R E L K Q Q M Q D I Q A E I D A H N D I F K S I D>

3290 3300 3310 3320 3330 3340 3350 3360
 GGAAACAGGC AGAAGATGGT AAAAGCTTTG GGAAATTCTG AAGAGGCTAC TATGCTTCAA CATCGACTGG ATGATATGAA
 G N R Q K M V K A L G N S E E A T M L Q H R L D D M N>

3370 3380 3390 3400 3410 3420 3430 3440
 CCAAAGATGG AATGACTTAA AAGCAAATC TGCTAGCATC AGGGCCCAT TGGAGGCCAG CGCTGAGAAG TGAACAGGT
 Q R W N D L K A K S A S I R A H L E A S A E K W N R>

3450 3460 3470 3480 3490 3500 3510 3520
 TGCTGATGTC CTTAGAAGAA CTGATCAAAT GGCTGAATAT GAAAGATGAA GAGCTTAAGA AACAAATGCC TATTGGAGGA
 L L M S L E E L I K W L N M K D E E L K K Q M P I G G>

3530 3540 3550 3560 3570 3580 3590 3600
 GATGTTCCAG CTTACAGCT CCAGTATGAC CATTGTAAGG CCCTGAGACG GGAGTTAAAG GAGAAAGAAT ATTCTGCTCT
 D V P A L Q L Q Y D H C K A L R R E L K E K E Y S V L>

3610 3620 3630 3640 3650 3660 3670 3680
 GAATGCTGTC GACCAGGCC GAGTTTCTT GGCTGATCAG CCAATTGAGG CCCCTGAAGA GCCAAGAAGA AACCTACAAT
 N A V D Q A R V F L A D Q P I E A P E E P R R N L Q>

3690 3700 3710 3720 3730 3740 3750 3760
 CAAAAACAGA ATTAACCTCT GAGGAGAGAG CCCAAAGAT TGCCAAAGCC ATGCGCAAAC AGTCTTCTGA AGTCAAAGAA
 S K T E L T P E E R A Q K I A K A M R K Q S S E V K E>

3770 3780 3790 3800 3810 3820 3830 3840
 AAATGGGAAA GTCTAAATGC TGTAAC TAGC AATTGGCAA AGCAAGTGGA CAAGGCATTG GAGAAACTCA GAGACCTGCA
 K W E S L N A V T S N W Q K Q V D K A L E K L R D L Q>

3850 3860 3870 3880 3890 3900 3910 3920
 GGGAGCTATG GATGACCTGG ACGCTGACAT GAAGGAGGCA GAGTCCGTGC GGAATGGCTG GAAGCCCGTG GGAGACTTAC
 G A M D D L D A D M K E A E S V R N G W K P V G D L>

3930 3940 3950 3960 3970 3980 3990 4000
 TCATTGACTC GCTGCAGGAT CACATTGAAA AAATCATGGC ATTTAGAGAA GAAATTGCAC CAATCAACTT TAAAGTTAAA
 L I D S L Q D H I E K I M A F R E E I A P I N F K V K>

4010 4020 4030 4040 4050 4060 4070 4080
 ACGGTGAATG ATTTATCCAG TCAGCTGTCT CCACTTGACC TGCATCCCTC TCTAAAGATG TCTCGCCAGC TAGATGACCT

Figure 9 cont ...

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T V N D L S S Q L S P L D L H P S L K M S R Q L D D L>

4090 4100 4110 4120 4130 4140 4150 4160
TAATATGCGA TGGAACTTT TACAGGTTTC TGTGGATGAT CGCCTTAAAC AGCTTCAGGA AGCCACAGA GATTTGGAC
N M R W K L L Q V S V D D R L K Q L Q E A H R D F G>

4170 4180 4190 4200 4210 4220 4230 4240
CATCCTCTCA GCATTTTCTC TCTACGTCAG TCCAGCTGCC GTGGCAAAGA TCCATTTTCA ATAATAAAGT GCCCTATTAC
P S S Q H F L S T S V Q L P W Q R S I S H N K V P Y Y>

4250 4260 4270 4280 4290 4300 4310 4320
ATCAACCATC AAACACAGAC CACCTGTTGG GACCATCCTA AAATGACCGA ACTCTTTCAA TCCCTTGCTG ACCTGAATAA
I N H Q T Q T T C W D H P K M T E L F Q S L A D L N N>

4330 4340 4350 4360 4370 4380 4390 4400
TGTACGTTTT TCTGCCTACC GTACAGCAAT CAAAATCCGA AGACTACAAA AAGCACTATG TTTGGATCTC TTAGAGTTGA
V R F S A Y R T A I K I R R L Q K A L C L D L L E L>

4410 4420 4430 4440 4450 4460 4470 4480
GTACAACAAA TGAAATTTTC AAACAGCACA AGTTGAACCA AAATGACCGA CTCCTCAGTG TTCCAGATGT CATCAACTGT
S T T N E I F K Q H K L N Q N D Q L L S V P D V I N C>

4490 4500 4510 4520 4530 4540 4550 4560
CTGACAACAA CTTATGATGG ACTTGAGCAA ATGCATAAGG ACCTGGTCAA CGTTCCACTC TGTGTTGATA TGTGTCTCAA
L T T T Y D G L E Q M H K D L V N V P L C V D M C L N>

4570 4580 4590 4600 4610 4620 4630 4640
TTGGTTGCTC AATGTCTATG ACACGGGTCG AACTGGAAAA ATTAGAGTGC AGAGTCTGAA GATTGGATTA ATGTCTCTCT
W L L N V Y D T G R T G K I R V Q S L K I G L M S L>

4650 4660 4670 4680 4690 4700 4710 4720
CCAAAGGTCT CTTGGAAGAA AAATACAGAT ATCTCTTTAA GGAAGTTGCG GGGCCGACAG AAATGTGTGA CCAGAGGCAG
S K G L L E E K Y R Y L F K E V A G P T E M C D Q R Q>

4730 4740 4750 4760 4770 4780 4790 4800
CTGGGCCTGT TACTTCATGA TGCCATCCAG ATCCCCCGGC AGCTAGGTGA AGTAGCAGCT TTTGGAGGCA GTAATATTGA
L G L L L H D A I Q I P R Q L G E V A A F G G S N I E>

4810 4820 4830 4840 4850 4860 4870 4880
GCCTAGTGTT CGCAGCTGCT TCCAACAGAA TAACAATAAA CCAGAAATAA GTGTGAAAGA GTTATAGAT TGGATGCATT
P S V R S C F Q Q N N N K P E I S V K E F I D W M H>

4890 4900 4910 4920 4930 4940 4950 4960
TGGAACCACA GTCCATGGTT TGGCTCCCAG TTTTACATCG AGTGGCAGCA GCGGAGACTG CAAAACATCA GGCCAAATGC
L E P Q S M V W L P V L H R V A A A E T A K H Q A K C>

4970 4980 4990 5000 5010 5020 5030 5040
AACATCTGTA AAGAATGTCC AATTGTCCGG TTCAGGTATA GAAGCCTTAA GCATTTTAAAC TATGATGTCT GCCAGAGTTG
N I C K E C P I V G F R Y R S L K H F N Y D V C Q S C>

5050 5060 5070 5080 5090 5100 5110 5120
TTTCTTTTCG GGTGGAACAG CAAAAGGTCA CAAATTACAT TACCCAATGG TGAATATTG TATACCTACA ACATCTGGGG
F F S G R T A K G H K L H Y P M V E Y C I P T T S G>

5130 5140 5150 5160 5170 5180 5190 5200
AAGATGTACG AGACTTCACA AAGGTACTTA AGAACAAGTT CAGGTCGAAG AAGTACTTTG CCAAACACCC TCGACTTGGT
E D V R D F T K V L K N K F R S K K Y F A K H P R L G>

5210 5220 5230 5240 5250 5260 5270 5280
TACCTGCCTG TCCAGACAGT TCTTGAAGGT GACAACTTAG AGACTCCTAT CACACTCATC AGTATGTGGC CAGAGACTA
Y L P V Q T V L E G D N L E T P I T L I S M W P E H Y>

5290 5300 5310 5320 5330 5340 5350 5360
TGACCCCTCA CAATCTCTC AACTGTTTCA TGATGACACC CATTCAAGAA TAGAACAATA TGCCACACGA CTGGCCAGAA
D P S Q S P Q L F H D D T H S R I E Q Y A T R L A Q>

5370 5380 5390 5400 5410 5420 5430 5440
TGGAAAGGAC TAATGGGTCT TTTCTCACTG ATAGCAGCTC CACCACAGGA AGTGTGGAAG ACGAGCACGC CCTCATCCAG

Figure 9 cont ...


```

M E R T N G S F L T D S S S T T G S V E D E H A L I Q>
      5450      5460      5470      5480      5490      5500      5510      5520
CAGTATTGCC AAACACTCGG AGGAGAGTCC CCAGTGAGCC AGCCGCAGAG CCCAGCTCAG ATCCTGAAGT CAGTAGAGAG
Q Y C Q T L G G E S P V S Q P Q S P A Q I L K S V E R>

      5530      5540      5550      5560      5570      5580      5590      5600
GGAAGAACGT GGAGAACTGG AGAGGATCAT TGCTGACCTG GAGGAAGAAC AAAGAAATCT ACAGGTGGAG TATGAGCAGC
E E R G E L E R I I A D L E E E Q R N L Q V E Y E Q>

      5610      5620      5630      5640      5650      5660      5670      5680
TGAAGGACCA GCACCTCCGA AGGGGGCTCC CTGTCGGTTC ACCGCCAGAG TCGATTATAT CTCCCCATCA CAGTCTGAG
L K D Q H L R R G L P V G S P P E S I I S P H H T S E>

      5690      5700      5710      5720      5730      5740      5750      5760
GATTCAGAAC TTATAGCAGA AGCAAACTC CTCAGGCAGC ACAAAGGTCG GCTGGAGGCT AGGATGCAGA TTTTGAAGA
D S E L I A E A K L L R Q H K G R L E A R M Q I L E D>

      5770      5780      5790      5800      5810      5820      5830      5840
TCACAATAAA CAGCTGGAGT CTCAGCTCCA CCGCCTCCGA CAGCTGCTGG AGCAGCCTGA ATCTGATTCC CGAATCAATG
H N K Q L E S Q L H R L R Q L L E Q P E S D S R I N>

      5850      5860      5870      5880      5890      5900      5910      5920
GTGTTTCCCC ATGGGCTTCT CCTCAGCATT CTGCACTGAG CTACTCGCTT GATCCAGATG CCTCCGGCCC ACAGTTCCAC
G V S P W A S P Q H S A L S Y S L D P D A S G P Q F H>

      5930      5940      5950      5960      5970      5980      5990      6000
CAGGCAGCGG GAGAGGACCT GCTGGCCCCA COGCACGACA CCAGCACGGA TCTCACGGAG GTCATGGAGC AGATTACAG
Q A A G E D L L A P P H D T S T D L T E V M E Q I H S>

      6010      6020      6030      6040      6050
CACGTTTCCA TCTTGCTGCC CAAATGTTCC CAGCAGGCCA CAGGCAATGT AATCACTAG
T F P S C C P N V P S R P Q A M *>

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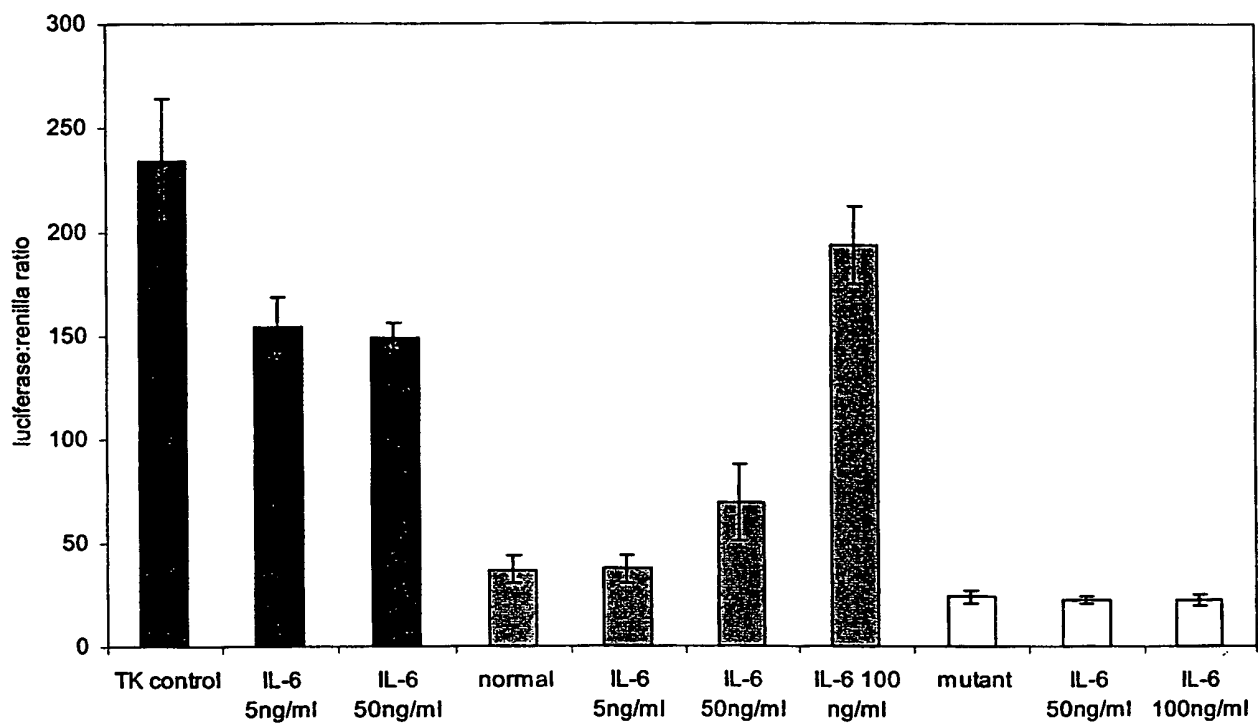



Figure 10

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 00/03800

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/85 C12N15/12 A01K67/027 C07K14/47 C07K16/18
A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Sequence HS91J24, human DNA sequence from clone 91J24 on chromosome 6q24. ; Contains part of utrophin gene. AccNo AL204474, 18 June 1998 (1998-06-18) TUBBY B: XP002160374 nucleotides 87734-88930 are identical to the sequence in figure 1 and have 83% sequence identity with the sequence in figure 2 ---	2-9, 23-28
P, X	EP 1 033 401 A (GENSET SA) 6 September 2000 (2000-09-06) SEQ ID NO:5164, claim 13 --- -/-	23-28

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

15 February 2001

Date of mailing of the international search report

28/02/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Cupido, M

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International Application No

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